(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 414 178 A2**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: **90115877.4**

(22) Date of filing: **18.08.90**

(51) Int. Cl.5: **C12N 15/12**, C12N 15/62, C12N 15/13, C12P 21/02, C12Q 1/70, A61K 37/02, G01N 33/569

The title of the invention has been amended (Guidelines for Examination in the EPO, A-III, 7.3).

The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(30) Priority: **23.08.89 US 397782**

(43) Date of publication of application: **27.02.91 Bulletin 91/09**

(84) Designated Contracting States: **AT BE CH DE DK ES FR GB GR IT LI LU NL SE**

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(54) Non-human primate CD4 polypeptides and human CD4 molecules capable of being glycosylated.

(57) The invention relates to substantially pure non-human primate CD4, and fragments thereof which bind to HIV or SIV gp120. The invention also relates to gp120 binding molecules related to human CD4 but which may exist in glycosylated form.

The invention also relates to fusion proteins which comprise the CD4 molecules of the invention, or fragments thereof, and an immunoglobulin light or heavy chain, wherein the variable region of the light or heavy chain has been replaced with CD4 or fragment thereof which is capable of binding to gp120. The invention also relates to fusion proteins comprising the CD4 molecules of the invention and a cytotoxic polypeptide.

The invention also relates to an immunoglobulin-like molecules comprising the fusion proteins of the invention together with an immunoglobulin light or heavy chain.

The invention also relates to methods of treating HIV or SIV infection comprising administering the CD4 molecules of the invention, glycoproteins, fragments thereof, fusion proteins or immunoglobulin-like molecules of the invention to an animal.

The invention also relates to assays for HIV or SIV comprising contacting a sample suspected of containing HIV or SIV gp120 with the CD4 molecules of the invention, fragments thereof, glycoproteins, immunoglobulin-like molecules, or fusion proteins of the invention, and detecting whether a complex is formed.

The invention also relates to nucleic acid molecules which specify the proteins, glycoproteins and fusion proteins of the invention as well as vectors and transformed hosts.

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NON-HUMAN PRIMATE CD4 POLYPEPTIDES, HUMAN CD4 MOLECULES CAPABLE OF GLYCOSYLATION, FRAGMENTS THEREOF, FUSION PROTEINS THEREOF, GENETIC SEQUENCES, AND THE USE THEREOF

FIELD OF THE INVENTION

The invention is in the field of recombinant genetics and pharmaceutical compositions.

BACKGROUND OF THE INVENTION

The human and simian immunodeficiency viruses HIV and SIV are the causative agents of Acquired Immune Deficiency Syndrome (AIDS) and Simian Immunodeficiency Syndrome (SIDS), respectively. See Curren, J. et al. , Science 329 : 1359-1357 (1985); Weiss, R. et al. , Nature 324 :572-575 (1986). The HIV virus contains an envelope glycoprotein, gp120 which binds to the CD4 protein present on the surface of helper T lymphocytes, macrophages and other cells. Dalgleish et al. Nature , 312 :763 (1984). After the gp120 binds to CD4, virus entry is facilitated by an envelope-mediated fusion of the viral target cell membranes.

During the course of infection, the host organism develops antibodies against viral proteins, including the major envelope glycoproteins gp120 and gp41. Despite this humoral immunity, the disease progresses, resulting in a lethal immunosuppression characterized by multiple opportunistic infections, parasitemia, dementia and death. The failure of host anti-viral antibodies to arrest the progression of the disease represents one of the most vexing and alarming aspects of the infection, and augurs poorly for vaccination efforts based upon conventional approaches.

Two factors may play a role in the inefficacy of the humoral response to immunodeficiency viruses. First, like other RNA viruses (and like retroviruses in particular), the immunodeficiency viruses show a high mutation rate which allows antigenic variation to progress at a high rate in response to host immune surveillance. Second, the envelope glycoproteins themselves are heavily glycosylated molecules presenting few epitopes suitable for high affinity antibody binding. The poorly antigenic, "moving" target which the viral envelope presents, allows the host little opportunity for restricting viral infection by specific antibody production.

Cells infected by the HIV virus express the gp120 glycoprotein on their surface. Gp120 mediates fusion events among $CD4^+$ cells via a reaction similar to that by which the virus enters the uninfected cell, leading to the formation of short-lived multinucleated giant cells. Syncytium formation is dependent on a direct interaction of the gp120 envelope glycoprotein with the CD4 protein. Dalgleish et al. , supra , Klatzmann, D. et al. , Nature 312 :763 (1984); McDougal, J.S. et al. Science , 231 :382 (1986); Sodroski, J. et al. , Nature , 322 :470 (1986); Lifson, J.D. et al. , Nature , 323 :725 (1986); Sodroski, J. et al. , Nature , 3 21 :412 (1986).

The human CD4 protein consists of a 372 amino acid extracellular region containing four immunoglobulin-like domains, a membrane spanning domain, and a charged intracellular region of 40 amino acid residues. Maddon, P. et al. , Cell 42 :93 (1985); Clark, S. et al. , Proc. Natl. Acad. Sci. (USA) 84 :1649 (1987).

Evidence that CD4-gp120 binding is responsible for viral infection of cells bearing the CD4 antigen includes the finding that a specific complex is formed between gp120 and CD4. McDougal et al. , supra . Other workers have shown that cell lines, which were non-infective for HIV, were converted to infectable cell lines following transfection and expression of the human CD4 cDNA gene. Maddon et al. , Cell 47 :333-348 (1986). PCT Application Publication Nos. WO 88/01304 (1988) and WO89/01940 (1989) disclose that soluble forms of human CD4 comprising the immunoglobulin-like binding domains are useful for the treatment or prophylaxis of HIV infections.

In contrast to the majority of antibody-envelope interactions, the receptor-envelope interaction is characterized by a high affinity (K_a = 10^8l/mole) immutable association. Moreover, the affinity of the virus for human CD4 is at least 3 orders of magnitude higher than the affinity of human CD4 for its putative endogenous ligand, the MHC class II antigens.

A number of workers have disclosed methods for preparing hybrid proteins. For example, Murphy, United States Patent 4,675,382 (1987), discloses the use of recombinant DNA techniques to make hybrid protein molecules by forming the desired fused gene coding for a hybrid protein of diphtheria toxin and a polypeptide ligand such as a hormone, followed by expression of the fused gene.

Many workers have prepared monoclonal antibodies (Mabs) by recombinant DNA techniques. Mon-

oclonal antibodies are highly specific well-characterized molecules in both primary and tertiary structure. They have been widely used for in vitro immunochemical characterization and quantitation of antigens. Genes for heavy and light chains have been introduced into appropriate hosts and expressed, followed by reaggregation of the individual chains into functional antibody molecules (see, for example, Munro, Nature 312 :597 (1984); Morrison, S.L., S cience 229 :1202 (1985); Oi et al ., Biotechniques 4 :214 (1986); Wood et al ., Nature 314 :446-449 (1985)). Light- and heavy-chain variable regions have been cloned and expressed in foreign hosts wherein they maintained their binding ability (Moore et al ., European Patent Application 0088994 (published September 21, 1983)).

Chimeric or hybrid antibodies have also been prepared by recombinant DNA techniques. Oi and Morrison, Biotechniques 4 :214 (1986) describe a strategy for producing such chimeric antibodies which include a chimeric human IgG anti-leu3 antibody.

Gascoigne, N.R.J., et al. , Proc. Natl. Acad. Sci. (USA) 84 :2936-2940 (1987) disclose the preparation of a chimeric gene construct containing a T-cell receptor α-chain variable (V) domain and the constant (C) region coding sequence of an immunoglobulin ,2a molecule. Cells transfected with the chimeric gene synthesize a protein product that expresses immunoglobulin and T-cell receptor antigenic determinants as well as protein A binding sites. This protein associates with a normal chain to form an apparently normal tetrameric (H_2L_2, where H = heavy and L = light) immunoglobulin molecule that is secreted.

Sharon, J., et al ., Nature 309 :54 (1984), disclose construction of a chimeric gene encoding the variable (V) region of a mouse heavy chain specific for the hapten azophenylarsonate and the constant (C) region of a mouse kappa light chain (V_HC_K). This gene was introduced into a mouse myeloma cell line. The chimeric gene was expressed to give a protein which associated with light chains secreted from the myeloma cell line to give an antibody molecule specific for azophenylarsonate.

Morrison, Science 229 :1202 (1985), discloses that variable light- or variable heavy-chain regions can be attached to a non-Ig sequence to create fusion proteins. This article states that the potential uses for the fusion proteins are three: (1) to attach antibody specifically to enzymes for use in assays: (2) to isolate non-Ig proteins by antigen columns; and (3) to specifically deliver toxic agents.

Recent techniques for the stable introduction of immunoglobulin genes into myeloma cells (Banerji, J., et al ., Cell 33 :729-740 (1983); Potter, H., et al ., Proc. Natl. Acad. Sci. (USA) 81 :7161-7165 (1984)), coupled with detailed structural information, have permitted the use of in vitro DNA methods such as mutagenesis, to generate recombinant antibodies possessing novel properties.

PCT Application WO87/02671 discloses methods for producing genetically engineered antibodies of desired variable region specificity and constant region properties through gene cloning and expression of light and heavy chains. The mRNA from cloned hybridoma B cell lines which produce monoclonal antibodies of desired specificity is isolated for cDNA cloning. The generation of light and heavy chain coding sequences is accomplished by excising the cloned variable regions and ligating them to light or heavy chain module vectors. This gives cDNA sequences which code for immunoglobulin chains. The lack of introns allows these cDNA sequences to be expressed in prokaryotic hosts, such as bacteria, or in lower eukaryotic hosts, such as yeast.

The generation of chimeric antibodies in which the antigen-binding portion of the immunoglobulin is fused to other moieties has been demonstrated. Examples of non-immunoglobulin genes fused to antibodies include Staphylococcus aureus nuclease, the mouse oncogene c-myc . and the Klenow fragment of E. coli DNA polymerase I (Neuberger, M.S., et al ., Nature 312 :604-612 (1984); Neuberger, M.S., Trends in Biochemical Science , 347-349 (1985)). European Patent Application 120.694 discloses the genetic engineering of the variable and constant regions of an immunoglobulin molecule that is expressed in E. coli host cells. It is further disclosed that the immunoglobulin molecule may be synthesized by a host cell with another peptide moiety attached to one of the constant domains. Such peptide moieties are described as either cytotoxic or enzymatic. The application and the examples describe the use of a lambda-like chain derived from a monoclonal antibody which binds to 4-hydroxy-3-nitrophenyl (NP) haptens.

European Patent Application 125,023 relates to the use of recombinant DNA techniques to produce immunoglobulin molecules that are chimeric or otherwise modified. One of the uses described for these immunoglobulin molecules is for whole-body diagnosis and treatment by injection of the antibodies directed to specific target tissues. The presence of the disease can be determined by attaching a suitable label to the antibodies, or the diseased tissue can be attacked by carrying a suitable drug with the antibodies. The application describes antibodies engineered to aid the specific delivery of an agent as "altered antibodies."

PCT Application WO83/101533 describes chimeric antibodies wherein the variable region of an immunoglobulin molecule is linked to a portion of a second protein which may comprise the active portion of an enzyme.

Boulianne et al ., Nature 312 :643 (1984) constructed an immunoglobulin gene in which the DNA

segments that encode mouse variable regions specific for the hapten trinitrophenol (TNP) are joined to segments that encode human mu and kappa regions. These chimeric genes were expressed to give functional TNP-binding chimeric IgM.

Morrison et al ., P.N.A.S. (USA) 81 :6851 (1984), disclose a chimeric molecule utilizing the heavy-chain variable region exons of an anti-phosphoryl choline myeloma protein G, which were joined to the exons of either human kappa light-chain gene. The genes were transfected into mouse myeloma cell lines, generating transformed cells that produced chimeric mouse-human IgG with antigen-binding function.

PCT Application Publication No. WO89/02922 (1989), discloses chimeric antibody molecules comprising human CD4. Such chimeric antibody molecules may be administered to a subject infected with HIV to treat the HIV infection.

Despite the progress that has been achieved on determining the mechanism of HIV infection, a need continues to exist for methods of treating HIV viral infections.

SUMMARY OF THE INVENTION

The invention relates to a nucleic acid molecule specifying non-human primate CD4, or an HIV or SIV gp120 binding fragment thereof.

In particular, the invention relates to a nucleic acid molecule specifying rhesus monkey CD4 comprising the following DNA sequence:

```
   1  ATGAACCGGGGAATCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTACTCCCA
 -25  MetAsnArgGlyIleProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro

      GCAGTCACCCAGGGAAAGAAAGTGGTGCTGGGCAAGAAAGGGGATACAGTGGAACTGACC   120
      AlaValThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr    15

 121  TGTACAGCTTCGCAGAAGAAGAACACACAATTCCACTGGAAAAACTCCAACCAGATAAAG
  16  CysThrAlaSerGlnLysLysAsnThrGlnPheHisTrpLysAsnSerAsnGlnIleLys
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                ATTCTGGGAATTCAGGGTCTCTTCTTAACTAAAGGTCCATCCAAGCTGAGCGATCGTGCT   240
                IleLeuGlyIleGlnGlyLeuPheLeuThrLysGlyProSerLysLeuSerAspArgAla    55

  241           GACTCAAGAAAAAGCCTTTGGGACCAAGGATGCTTTTCCATGATCATCAAGAATCTTAAG
   56           AspSerArgLysSerLeuTrpAspGlnGlyCysPheSerMetIleIleLysAsnLeuLys

                ATAGAAGACTCAGATACTTACATCTGTGAAGTGGAGAACAAGAAGGAGGAGGTGGAATTG   360
                IleGluAspSerAspThrTyrIleCysGluValGluAsnLysLysGluGluValGluLeu    95

  361           CTGGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTGAGGGGCAAAGCCTGACC
   96           LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGluGlyGlnSerLeuThr

                CTGACCTTGGAGAGCCCCCCTGGTAGTAGCCCCTCAGTGAAATGTAGGAGTCCAGGGGGT   480
                LeuThrLeuGluSerProProGlySerSerProSerValLysCysArgSerProGlyGly   135

  481           AAAAACATACAGGGGGGGAGGACCATCTCTGTGCCTCAGCTGGAGCGCCAGGATAGTGGC
  136           LysAsnIleGlnGlyGlyArgThrIleSerValProGlnLeuGluArgGlnAspSerGly

                ACCTGGACATGCACCGTCTCGCAGGACCAGAAGACGGTGGAGTTCAAAATAGACATCGTG   600
                ThrTrpThrCysThrValSerGlnAspGlnLysThrValGluPheLysIleAspIleVal   175

  601           GTGCTAGCTTTCCAGAAGGCCTCCAGCACAGTCTATAAGAAAGAGGGGGAACAGGTGGAG
  176           ValLeuAlaPheGlnLysAlaSerSerThrValTyrLysLysGluGlyGluGlnValGlu

                TTCTCCTTCCCACTCGCCTTTACACTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGG   720
                PheSerPheProLeuAlaPheThrLeuGluLysLeuThrGlySerGlyGluLeuTrpTrp   215

  721           CAGGCGGAGAGGGCCTCCTCCTCCAAGTCTTGGATTACCTTCGACCTGAAGAACAAGGAA
  216           GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu

                GTGTCTGTAAAACGGGTTACCCAGGACCCCAAGCTCCAGATGGGCAAGAAGCTCCCGCTC   840
                ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu   255

  841           CACCTCACCCTGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACGCTGGCC
  256           HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla

                CTTGAAGCGAAAACAGGAAAGTTGCATCAGGAAGTGAACCTCGTGGTGATGAGAGCCACT   960
                LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr   295
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 961  CAGTTCCAGGAAAATTTGACCTGTGAAGTGTGGGGACCCACCTCCCCTAAGCTGACGCTG
 296  GlnPheGlnGluAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuThrLeu

      AGCTTGAAACTGGAGAACAAGGGGGCAACGGTCTCGAAGCAGGCGAAGGCGGTGTGGGTG   1080
      SerLeuLysLeuGluAsnLysGlyAlaThrValSerLysGlnAlaLysAlaValTrpVal   335

1081  CTGAACCCTGAGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTA
 336  LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu

      GAATCCAACATCAAGGTTGTGCCCACATGGCCCACCCCGGTGCAGCCAATGGCCCTGATT   1200
      GluSerAsnIleLysValValProThrTrpProThrProValGlnProMetAlaLeuIle   375

1201  GTGCTGGGGGGCGTTGCGGGCCTCCTGCTTTTCACTGGGCTAGGCATCTTCTTCTGTGTC
 376  ValLeuGlyGlyValAlaGlyLeuLeuLeuPheThrGlyLeuGlyIlePhePheCysVal

      AGGTGCCGGCATCGAAGGCGTCAAGCAGAGCGGATGTCTCAGATCAAGAGACTCCTCAGT   1320
      ArgCysArgHisArgArgArgGlnAlaGluArgMetSerGlnIleLysArgLeuLeuSer   415

1321  GAAAAGAAGACCTGCCAGTGCCCTCACCGGTTTCAGAAGACATGTAGCCCCATTTGA  1377
 416  GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIleEnd   433
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or a degenerate variant thereof.

The invention also relates to a nucleic acid molecule specifying a soluble non-human primate CD4 fragment. In particular, the invention to a soluble rhesus CD4 fragment (domain I) which binds HIV or SIV gp120 comprising the following DNA sequence:

```
   1  ATGAACCGGGGAATCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTACTCCCA
 -25  MetAsnArgGlyIleProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro

      GCAGTCACCCAGGGAAAGAAAGTGGTGCTGGGCAAGAAAGGGGATACAGTGGAACTGACC   120
      AlaValThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr    15
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121  TGTACAGCTTCGCAGAAGAAGAACACACAATTCCACTGGAAAAACTCCAACCAGATAAAG
 16  CysThrAlaSerGlnLysLysAsnThrGlnPheHisTrpLysAsnSerAsnGlnIleLys

     ATTCTGGGAATTCAGGGTCTCTTCTTAACTAAAGGTCCATCCAAGCTGAGCGATCGTGCT   240
     IleLeuGlyIleGlnGlyLeuPheLeuThrLysGlyProSerLysLeuSerAspArgAla    55

241  GACTCAAGAAAAAGCCTTTGGGACCAAGGATGCTTTTCCATGATCATCAAGAATCTTAAG
 56  AspSerArgLysSerLeuTrpAspGlnGlyCysPheSerMetIleIleLysAsnLeuLys

     ATAGAAGACTCAGATACTTACATCTGTGAAGTGGAGAACAAGAAGGAGGAGGTGGAATTG   360
     IleGluAspSerAspThrTyrIleCysGluValGluAsnLysLysGluGluValGluLeu    95

361  CTGGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTT
 96  LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu
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or a degenerate variant thereof.

The invention also relates to a nucleic acid molecule specifying chimpanzee CD4, comprising the following DNA sequence:

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  1  ATGAACCGGGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCACTCCTCCCA
-25  MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro

     GCAGCCACTCAGGGAAAGAAAGTGGTGCTGGGCAAGAAAGGGGACACAGTGGAACTGACC   120
     AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr    15

121  TGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGACAAAG
 16  CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnThrLys

     ATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGTT   240
     IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgVal    55

241  GACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTTACCCTGATCATCAAGAATCTTAAG
 56  AspSerArgArgSerLeuTrpAspGlnGlyAsnPheThrLeuIleIleLysAsnLeuLys
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                ATAGAAGACTCAGATACTTACATCTGTGAAGTGGGGGACCAGAAGGAGGAGGTGCAATTG   360
                IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu    95

 361  CTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACC
  96  LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGlnGlyGlnSerLeuThr

                CTGACCTTGGAGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGT   360
                LeuThrLeuGluSerProProGlySerSerProSerValGlnCysArgSerProArgGly   135

 481  AAAAACATACAGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGC
 136  LysAsnIleGlnGlyGlyLysThrLeuSerValSerGlnLeuGluLeuGlnAspSerGly

                ACCTGGACATGCACTGTCTTGCAGAACCAGAAGAAAGTGGAGTTCAAAATAGACATCGTG   600
                ThrTrpThrCysThrValLeuGlnAsnGlnLysLysValGluPheLysIleAspIleVal   175

 601  GTGCTAGCTTTCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGGAG
 176  ValLeuAlaPheGlnLysAlaSerSerIleValTyrLysLysGluGlyGluGlnValGlu

                TTCTCCTTCCCACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGG   720
                PheSerPheProLeuAlaPheThrValGluLysLeuThrGlySerGlyGluLeuTrpTrp   215

 721  CAGGCGGAGAGGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAA
 216  GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu

                GTGTCTGTAAAACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTC   840
                ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu   255

 841  CACCTCACCCTGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACCCTGGCC
 256  HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla

                CTTGAAGCGAAAACAGGAAAGTTGCATCAGGAAGTGAACCTCGTGGTGATGAGAGCCACT   840
                LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr   295

 961  CAGCTCCAGAAAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTG
 296  GlnLeuGlnLysAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuMetLeu

                AGCTTGAAACTGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGGCGGTGTGGGTG  1080
                SerLeuLysLeuGluAsnLysGluAlaLysValSerLysArgGluLysAlaValTrpVal   335
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```
1081  CTGAACCCTGAGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTG
 336  LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu

                                                                    1200
      GAATCCAACATCAAGGTTCTGCCCACATGGTCCACCCCGGTGCAGCCAATGGCCCTGATT
      GluSerAsnIleLysValLeuProThrTrpSerThrProValGlnProMetAlaLeuIle   375

1201  GTGCTGGGGGGCGTCGCCGGCCTCCTGCTTTTCATTGGGCTAGGCATCTTCTTCTGTGTC
 376  ValLeuGlyGlyValAlaGlyLeuLeuLeuPheIleGlyLeuGlyIlePhePheCysVal

                                                                    1320
      AGGTGCCGGCACCGAAGGCGCCAAGCACAGCGGATGTCTCAGATCAAGAGACTCCTCAGT
      ArgCysArgHisArgArgArgGlnAlaGlnArgMetSerGlnIleLysArgLeuLeuSer   415

1321  GAGAAGAAGACCTGCCAGTGCCCTCACCGGTTTCAGAAGACATGTAGCCCCATTTGA  1377
 416  GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIleEnd   433
```

or a degenerate variant thereof.

The invention also relates to a nucleic acid molecule specifying a soluble chimpanzee CD4 (domain I) which binds HIV or SIV gp120, comprising the following DNA sequence:

```
   1  ATGAACCGGGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCACTCCTCCCA
-25  MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro

      GCAGCCACTCAGGGAAAGAAAGTGGTGCTGGGCAAGAAAGGGGACACAGTGGAACTGACC   120
      AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr    15

 121  TGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGACAAAG
  16  CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnThrLys

      ATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGTT   240
      IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgVal    55

 241  GACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTTACCCTGATCATCAAGAATCTTAAG
  56  AspSerArgArgSerLeuTrpAspGlnGlyAsnPheThrLeuIleIleLysAsnLeuLys

      ATAGAAGACTCAGATACTTACATCTGTGAAGTGGGGGACCAGAAGGAGGAGGTGCAATTG   360
      IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu    95

 361  CTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTT
  96  LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu
```

or a degenerate variant thereof.

The invention also relates to a nucleic acid molecule specifying chimpanzee CD4 with the cytoplasmic domain, comprising the following DNA sequence:

```
    1  ATGAACCGGGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCACTCCTCCCA
-25  MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro

     GCAGCCACTCAGGGAAAGAAAGTGGTGCTGGGCAAGAAAGGGGACACAGTGGAACTGACC   120
     AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr    15

121  TGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGAYAAAG
 16  CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnThrLys
                                                              Ile

     ATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGYT   240
     IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgVal    55
                                                                 Ala

241  GACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTTMCCCTGATCATCAAGAATCTTAAG
 56  AspSerArgArgSerLeuTrpAspGlnGlyAsnPheThrLeuIleIleLysAsnLeuLys
                                                 Pro

     ATAGAAGACTCAGATACTTACATCTGTGAAGTGGGGGACCAGAAGGAGGAGGTGCAATTG   360
     IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu    95

361  CTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACC
 96  LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGlnGlyGlnSerLeuThr

     CTGACCTTGGAGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGT   360
     LeuThrLeuGluSerProProGlySerSerProSerValGlnCysArgSerProArgGly   135
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481  AAAAACATACAGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGC
136  LysAsnIleGlnGlyGlyLysThrLeuSerValSerGlnLeuGluLeuGlnAspSerGly

     ACCTGGACATGCACTGTCTTGCAGAACCAGAAGAAAGTGGAGTTCAAAATAGACATCGTG   600
     ThrTrpThrCysThrValLeuGlnAsnGlnLysLysValGluPheLysIleAspIleVal   175

601  GTGCTAGCTTTCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGGAG
176  ValLeuAlaPheGlnLysAlaSerSerIleValTyrLysLysGluGlyGluGlnValGlu

     TTCTCCTTCCCACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGG   720
     PheSerPheProLeuAlaPheThrValGluLysLeuThrGlySerGlyGluLeuTrpTrp   215

721  CAGGCGGAGAGGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAA
216  GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu

     GTGTCTGTAAAACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTC   840
     ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu   255

841  CACCTCACCCTGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACCCTGGCC
256  HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla

     CTTGAAGCGAAAACAGGAAAGTTGCATCAGGAAGTGAACCTCGTGGTGATGAGAGCCACT   840
     LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr   295

961  CAGCTCCAGAAAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTG
296  GlnLeuGlnLysAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuMetLeu

     AGCTTGAAACTGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGGCGGTGTGGGTG  1080
     SerLeuLysLeuGluAsnLysGluAlaLysValSerLysArgGluLysAlaValTrpVal   335

1081 CTGAACCCTGAGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTG
336  LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu

     GAATCCAACATCAAGGTTCTGCCCACATGGTCCACCCCGGTGCAGCCAATGGCCCTGATT  1200
     GluSerAsnIleLysValLeuProThrTrpSerThrProValGlnProMetAlaLeuIle   375
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1201  GTGCTGGGGGGCGTCGCCGGCCTCCTGCTTTTCATTGGGCTAGGCATCTTCTTCTGTGTC
 376  ValLeuGlyGlyValAlaGlyLeuLeuPheIleGlyLeuGlyIlePhePheCysVal

     AGGTGCCGGCACCGAAGGCGCCAAGCASAGCGGATGTCTCAGATCAAGAGACTCCTCAGT  1320
     ArgCysArgHisArgArgArgGlnAlaGlnArgMetSerGlnIleLysArgLeuLeuSer   415
                                    Glu

1321  GAGAAGAAGACCTGCCAGTGCCCTCACCGGTTTCAGAAGACATGTAGCCCCATTTGA  1377
 416  GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIleEnd   433
```

wherein Y is C or T,
M is A or C, and
S is C or G;
or a degenerate variant thereof.

The invention also relates to a nucleic acid molecule specifying a chimpanzee CD4 fragment, comprising the following DNA sequence:

```
   1  ATGAACCGGGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCACTCCTCCCA
-25  MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro

     GCAGCCACTCAGGGAAAGAAAGTGGTGCTGGGCAAGAAAGGGGACACAGTGGAACTGACC   120
     AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr    15

121  TGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGAYAAAG
 16  CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnThrLys
                                                          Ile

     ATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGYT   240
     IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgVal    55
                                                              Ala

241  GACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTTMCCCTGATCATCAAGAATCTTAAG
 56  AspSerArgArgSerLeuTrpAspGlnGlyAsnPheThrLeuIleIleLysAsnLeuLys
                                        Pro

     ATAGAAGACTCAGATACTTACATCTGTGAAGTGGGGGACCAGAAGGAGGAGGTGCAATTG   360
     IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu    95

361  CTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTT
 96  LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu
```

wherein Y is C or T, and
M is A or C;
or a degenerate variant thereof.

The invention also relates to a nucleic acid molecule specifying a gp120 binding molecule capable of glycosylation which is related to human CD4 with the cytoplasmic domain, comprising the following DNA

sequence:

```
     1  ATGAACCGGGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCA
-25  MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro

     GCAGCCACTCAGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACC   120
     AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr    15

121  TGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGAYAAAG
 16  CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnThrLys
                                                              Ile

     ATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCT   240
     IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgAla    55

241  GACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTCMCCCTGATCATCAAGAATCTTAAG
 56  AspSerArgArgSerLeuTrpAspGlnGlyAsnPheThrLeuIleIleLysAsnLeuLys
                                                  Pro

     ATAGAAGACTCAGATACTTACATCTGTGAAGTGGAGGACCACAAGGAGGAGGTGCAATTG   360
     IleGluAspSerAspThrTyrIleCysGluValGluAspGlnLysGluGluValGlnLeu    95
```

```
361  CTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACC
 96  LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGlnGlyGlnSerLeuThr

     CTGACCTTGGAGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGT  360
     LeuThrLeuGluSerProProGlySerSerProSerValGlnCysArgSerProArgGly  135

481  AAAAACATACAGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGC
136  LysAsnIleGlnGlyGlyLysThrLeuSerValSerGlnLeuGluLeuGlnAspSerGly

     ACCTGGACATGCACTGTCTTGCAGAACCAGAAGAAGGTGGAGTTCAAAATAGACATCGTG  600
     ThrTrpThrCysThrValLeuGlnAsnGlnLysLysValGluPheLysIleAspIleVal  175

601  GTGCTAGCTTTCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGGAG  ----
176  ValLeuAlaPheGlnLysAlaSerSerIleValTyrLysLysGluGlyGluGlnValGlu

     TTCTCCTTCCCACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGG  720
     PheSerPheProLeuAlaPheThrValGluLysLeuThrGlySerGlyGluLeuTrpTrp  215

721  CAGGCGGAGAGGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAA
216  GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu

     GTGTCTGTAAAACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTC  840
     ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu  255

841  CACCTCACCCTGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACCCTGGCC
256  HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla

     CTTGAAGCGAAAACAGGAAAGTTGCATCAGGAAGTGAACCTGGTGGTGATGAGAGCCACT  840
     LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr  295

961  CAGCTCCAGAAAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTG
296  GlnLeuGlnLysAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuMetLeu

     AGCTTGAAACTGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGGCGGTGTGGGTG 1080
     SerLeuLysLeuGluAsnLysGluAlaLysValSerLysArgGluLysAlaValTrpVal  335
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```
1081  CTGAACCCTGAGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTG
 336  LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu

     GAATCCAACATCAAGGTTCTGCCCACATGGTCCACCCCGGTGCAGCCAATGGCCCTGATT  1200
     GluSerAsnIleLysValLeuProThrTrpSerThrProValGlnProMetAlaLeuIle   375

1201  GTGCTGGGGGGCGTCGCCGGCCTCCTGCTTTTCATTGGGCTAGGCATCTTCTTCTGTGTC
 376  ValLeuGlyGlyValAlaGlyLeuLeuLeuPheIleGlyLeuGlyIlePhePheCysVal

     AGGTGCCGGCACCGAAGGCGCCAAGCAGAGCGGATGTCTCAGATCAAGAGACTCCTCAGT  1320
     ArgCysArgHisArgArgArgGlnAlaGluArgMetSerGlnIleLysArgLeuLeuSer   415

1321  GAGAAGAAGACCTGCCAGTGCCCTCACCGGTTTCAGAAGACATGTAGCCCCATTTGA  1377
 416  GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIleEnd   433
```

wherein Y is C or T, and
M is A or C;
or a degenerate variant thereof;
with the proviso that both Y is not T and M is not C at the same time.

The invention also relates to a nucleic acid molecule specifying a gp120 binding molecule capable of glycosylation which is related to a human CD4 fragment, comprising the following DNA sequence:

```
   1  ATGAACCGGGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCA
 -25  MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro

     GCAGCCACTCAGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACC   120
     AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr    15
```

```
121  TGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGAYAAAG
 16  CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnThrLys
                                                            Ile

     ATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCT   240
     IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgAla    55

241  GACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTCMCCCTGATCATCAAGAATCTTAAG
 56  AspSerArgArgSerLeuTrpAspGlnGlyAsnPheThrLeuIleIleLysAsnLeuLys
                                          Pro

     ATAGAAGACTCAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTG   360
     IleGluAspSerAspThrTyrIleCysGluValGluAspGlnLysGluGluValGlnLeu    95

361  CTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTT
 96  LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu
```

wherein Y is C or T, and
M is A or C;
or a degenerate variant thereof;
with the proviso that both Y is not T and M is not C at the same time.

The invention also relates to a nucleic acid molecule specifying a fusion protein, comprising
1) a nucleic acid molecule specifying non-human primate CD4 or fragment thereof which binds HIV or SIV gp120, and
2) a nucleic acid molecule specifying an immunoglobulin light or heavy chain, wherein the nucleic acid molecule which specifies the variable region of said immunoglobulin chain has been replaced with the nucleic acid molecule specifying said non-human primate CD4 or fragment thereof.

The invention also relates to a nucleic acid molecule specifying a fusion protein, comprising
1) a nucleic acid molecule specifying non-human primate CD4, or fragment thereof which binds HIV or SIV gp120, linked to
2) a nucleic acid molecule specifying a cytotoxic polypeptide.

The invention also relates to vectors comprising the nucleic acid molecules of the invention.

The invention also relates to hosts transformed with the vectors of the invention. In particular, the invention relates to hosts which express complementary immunoglobulin light or heavy chains together with the expression product of said fusion protein nucleic acid molecule to give an immunoglobulin-like molecule which binds to HIV or SIV gp120.

The invention also relates to methods of producing non-human primate CD4, or fragment thereof which binds to HIV or SIV gp120, which comprises
cultivating in a nutrient medium under protein-producing conditions, a host strain transformed with a vector containing a nucleic acid molecule specifying a non-human primate CD4 or soluble fragment thereof which binds HIV or SIV gp120, said vector further comprising expression signals which are recognized by said host strain and direct expression of said non-human primate CD4 or fragment thereof, and
recovering the non-human primate CD4 or soluble fragment thereof so produced.

The invention also relates to a method of producing a fusion protein comprising non-human primate CD4, or fragment thereof which binds to gp120, and an immunoglobulin light or heavy chain, wherein the variable region of the immunoglobulin chain has been substituted with non-human primate CD4, or fragment thereof which binds to HIV or SIV gp120, which comprises
cultivating in a nutrient medium under protein-producing conditions, a host strain transformed with a vector specifying said fusion protein, said vector further comprising expression signals which are recognized by said host strain and direct expression of said fusion protein, and
recovering the fusion protein so produced.

In particular, the invention relates to a method of preparing a immunoglobulin-like molecule, wherein said host strain is a myeloma cell line which produces immunoglobulin light chains and said fusion protein comprises an immunoglobulin heavy chain of the class IgM, IgG1 or IgG3, wherein an immunoglobulin-like molecule comprising said fusion protein is produced. The invention also relates to a method of preparing an

immunoglobulin-like molecule, wherein said host produces immunoglobulin heavy chains of the class IgM, IgG1 and IgG3 together with said fusion protein comprising an immunoglobulin light chain to give an immunoglobulin-like molecule which binds to HIV or SIV gp120.

The invention also relates to substantially pure non-human primate CD4. In particular, the invention relates to substantially pure rhesus CD4 comprising the following amino acid sequence:

MetAsnArgGlyIleProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaValThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysAsnThrGlnPheHisTrpLysAsnSerAsnGlnIleLys
IleLeuGlyIleGlnGlyLeuPheLeuThrLysGlyProSerLysLeuSerAspArgAla
AspSerArgLysSerLeuTrpAspGlnGlyCysPheSerMetIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGluAsnLysLysGluGluValGluLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGluGlyGlnSerLeuThr
LeuThrLeuGluSerProProGlySerSerProSerValLysCysArgSerProGlyGly
LysAsnIleGlnGlyGlyArgThrIleSerValProGlnLeuGluArgGlnAspSerGly
ThrTrpThrCysThrValSerGlnAspGlnLysThrValGluPheLysIleAspIleVal

ValLeuAlaPheGlnLysAlaSerSerThrValTyrLysLysGluGlyGluGlnValGlu
PheSerPheProLeuAlaPheThrLeuGluLysLeuThrGlySerGlyGluLeuTrpTrp
GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu
ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu
HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla
LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr
GlnPheGlnGluAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuThrLeu
SerLeuLysLeuGluAsnLysGlyAlaThrValSerLysGlnAlaLysAlaValTrpVal
LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu
GluSerAsnIleLysValValProThrTrpProThrProValGlnProMetAlaLeuIle
ValLeuGlyGlyValAlaGlyLeuLeuLeuPheThrGlyLeuGlyIlePhePheCysVal
ArgCysArgHisArgArgArgGlnAlaGluArgMetSerGlnIleLysArgLeuLeuSer
GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIle.

The invention also relates to substantially pure chimpanzee CD4 comprising the following amino acid sequence:

MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnThrLys
IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgVal
AspSerArgArgSerLeuTrpAspGlnGlyAsnPheThrLeuIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGlnGlyGlnSerLeuThr
LeuThrLeuGluSerProProGlySerSerProSerValGlnCysArgSerProArgGly
LysAsnIleGlnGlyGlyLysThrLeuSerValSerGlnLeuGluLeuGlnAspSerGly
ThrTrpThrCysThrValLeuGlnAsnGlnLysLysValGluPheLysIleAspIleVal
ValLeuAlaPheGlnLysAlaSerSerIleValTyrLysLysGluGlyGluGlnValGlu
PheSerPheProLeuAlaPheThrValGluLysLeuThrGlySerGlyGluLeuTrpTrp
GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu
ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu
HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla
LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr
GlnLeuGlnLysAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuMetLeu

SerLeuLysLeuGluAsnLysGluAlaLysValSerLysArgGluLysAlaValTrpVal
LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu
GluSerAsnIleLysValLeuProThrTrpSerThrProValGlnProMetAlaLeuIle
ValLeuGlyGlyValAlaGlyLeuLeuLeuPheIleGlyLeuGlyIlePhePheCysVal
ArgCysArgHisArgArgArgGlnAlaGlnArgMetSerGlnIleLysArgLeuLeuSer
GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIle; or
the glycosylated derivative thereof.

The invention also relates to substantially pure non-human CD4 molecule comprising the following amino acid sequence:

MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGln-@-Lys
IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArg-#-
AspSerArgArgSerLeuTrpAspGlnGlyAsnPhe-$-LeuIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGlnGlyGlnSerLeuThr
LeuThrLeuGluSerProProGlySerSerProSerValGlnCysArgSerProArgGly
LysAsnIleGlnGlyGlyLysThrLeuSerValSerGlnLeuGluLeuGlnAspSerGly
ThrTrpThrCysThrValLeuGlnAsnGlnLysLysValGluPheLysIleAspIleVal
ValLeuAlaPheGlnLysAlaSerSerIleValTyrLysLysGluGlyGluGlnValGlu
PheSerPheProLeuAlaPheThrValGluLysLeuThrGlySerGlyGluLeuTrpTrp
GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu
ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu
HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla
LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr
GlnLeuGlnLysAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuMetLeu
SerLeuLysLeuGluAsnLysGluAlaLysValSerLysArgGluLysAlaValTrpVal
LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu
GluSerAsnIleLysValLeuProThrTrpSerThrProValGlnProMetAlaLeuIle
ValLeuGlyGlyValAlaGlyLeuLeuLeuPheIleGlyLeuGlyIlePhePheCysVal
ArgCysArgHisArgArgArgGlnAla-%-ArgMetSerGlnIleLysArgLeuLeuSer

GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIle,

wherein
-@- is Thr or Ile,
-#- is Val or Ala,
-$- is Thr or Pro, and
-%- is Gln or Glu; or
the glycosylated derivative thereof.

The invention also relates to a gp120 binding molecule related to human CD4 comprising the following amino acid sequence:

MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGln-@-Lys
IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgAla
AspSerArgArgSerLeuTrpAspGlnGlyAsnPhe-$-LeuIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGluAspGlnLysGluGluValGlnLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGlnGlyGlnSerLeuThr
LeuThrLeuGluSerProProGlySerSerProSerValGlnCysArgSerProArgGly
LysAsnIleGlnGlyGlyLysThrLeuSerValSerGlnLeuGluLeuGlnAspSerGly
ThrTrpThrCysThrValLeuGlnAsnGlnLysLysValGluPheLysIleAspIleVal
ValLeuAlaPheGlnLysAlaSerSerIleValTyrLysLysGluGlyGluGlnValGlu
PheSerPheProLeuAlaPheThrValGluLysLeuThrGlySerGlyGluLeuTrpTrp
GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu
ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu
HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla
LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr
GlnLeuGlnLysAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuMetLeu
SerLeuLysLeuGluAsnLysGluAlaLysValSerLysArgGluLysAlaValTrpVal
LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu
GluSerAsnIleLysValLeuProThrTrpSerThrProValGlnProMetAlaLeuIle
ValLeuGlyGlyValAlaGlyLeuLeuLeuPheIleGlyLeuGlyIlePhePheCysVal

ArgCysArgHisArgArgArgGlnAlaGluArgMetSerGlnIleLysArgLeuLeuSer
GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIle,

wherein
-@- is Thr or Ile, and
-$- is Thr or Pro; or
the glycosylated derivative thereof;
with the proviso that at least one of -@- and -$- is Thr.

The invention also relates to non-human primate CD4 fragments which binds to HIV or SIV gp120. Preferably, such non-human primate CD4 fragments are soluble in aqueous solution.

In particular, the invention relates to a soluble CD4 fragment which is derived from the rhesus monkey and comprises the following amino acid sequence:

MetAsnArgGlyIleProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaValThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysAsnThrGlnPheHisTrpLysAsnSerAsnGlnIleLys
IleLeuGlyIleGlnGlyLeuPheLeuThrLysGlyProSerLysLeuSerAspArgAla
AspSerArgLysSerLeuTrpAspGlnGlyCysPheSerMetIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGluAsnLysLysGluGluValGluLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu.

The invention also relates to a soluble chimpanzee CD4 fragment comprising the following amino acid sequence:

MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnThrLys
IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgVal
AspSerArgArgSerLeuTrpAspGlnGlyAsnPheThrLeuIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu.

The invention also relates to a gp120 binding molecule capable of glycosylation comprising the following amino acid sequence:

MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGln-@-Lys
IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArg-#-
AspSerArgArgSerLeuTrpAspGlnGlyAsnPhe-$-LeuIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu

wherein
-@- is Thr or Ile,
-#- is Val or Ala, and
-$- is Thr or Pro; or
the glycosylated derivative thereof.

The invention also relates to gp120 binding molecule capable of glycosylation related to human CD4 fragments. In particular, the invention relates to a glycosylated human CD4 fragment comprising the following amino acid sequence:

MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro

AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGln-@-Lys
IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgAla
AspSerArgArgSerLeuTrpAspGlnGlyAsnPhe-$-LeuIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGluAspGlnLysGluGluValGlnLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu

wherein
-@- is Thr or Ile, and
-$- Is Thr or Pro; or
the glycosylated derivative thereof;
with the proviso that at least one of -@- and -$- is Thr.

The invention also relates to fusion proteins, comprising non-human primate CD4 or gp120 binding molecules of the invention, or HIV or SIV binding fragments thereof, linked to a cytotoxic polypeptide.

The invention also relates to a fusion protein comprising non-human primate CD4 or gp120 binding molecules of the invention, or fragments thereof which are capable of binding to HIV or SIV gp120, fused at the C-terminus to a second protein which comprises an immunoglobulin heavy chain of the class IgM, IgG1 or IgG3, wherein the variable region of said heavy chain immunoglobulin has been replaced with CD4, or HIV gp120-binding fragment thereof.

The invention also relates to an immunoglobulin-like molecule, comprising:
(1) a fusion protein of non-human primate CD4 or fragment thereof which binds to HIV or SIV gp120 and an immunoglobulin heavy chain, linked to
(2) an immunoglobulin light chain.

The invention also relates to a fusion protein comprising non-human primate CD4 or gp120 binding molecules of the invention, or fragment thereof which binds to HIV or SIV gp120, fused at the C-terminus to a second protein comprising an immunoglobulin light chain where the variable region has been deleted.

The invention also relates to an immunoglobulin-like molecule comprising:
1) a fusion protein of non-human primate CD4 or gp120 binding molecule of the invention, or fragment thereof which binds to HIV or SIV gp120, and an immunoglobulin light chain, linked to
2) an immunoglobulin heavy chain.

The invention also relates to pharmaceutical compositions, comprising
1) a therapeutically effective amount of a non-human primate CD4, and
2) a pharmaceutically acceptable carrier.

The invention also relates to pharmaceutical compositions, comprising
1) a therapeutically effective amount of a soluble non-human CD4 fragment, and
2) a pharmaceutically acceptable carrier.

The invention also relates to pharmaceutical compositions comprising the proteins, glycoproteins, fusion proteins and immunoglobulin-like molecules of the invention.

The invention also relates to complexes between the substantially pure non-human primate CD4 and HIV or SIV gp120.

The invention also relates to complexes comprising the non-human primate CD4 fragments of the invention and HIV or SIV gp120.

The invention also relates to complexes comprising the fusion proteins and immunoglobulin-like molecules of the invention and HIV or SIV gp120.

The invention also relates to complexes between the gp120 binding molecules capable of glycosylation and HIV or SIV gp120.

The invention also relates to a method of treating HIV or SIV infections, comprising administering to an animal in need of such treatment a therapeutically effective amount of substantially pure non-human primate CD4, or a soluble fragment thereof.

The invention also relates to a method of treating HIV or SIV infections, comprising administering to an animal in need of such treatment a therapeutically effective amount one of the fusion proteins of the

invention.

The invention also relates to a method of treating HIV or SIV infections, comprising administering to an animal in need of such treatment a therapeutically effective amount one of the immunoglobulin-like molecules of the invention.

The invention also relates to a method of treating HIV or SIV infections, comprising administering to an animal in need of such treatment a therapeutically effective amount of the gp120 binding molecules of the invention.

The invention also relates to a method for the detection of HIV or SIV gp120 in a sample, comprising:

(a) contacting a sample suspected of containing HIV or SIV gp120 with the fusion protein or immunoglobulin-like molecule of the invention; and

(b) detecting whether a complex is formed.

The invention also relates to a method for the detection of HIV or SIV gp120 in a sample, comprising

(a) contacting a sample suspected of containing HIV or SIV gp120 with substantially pure non-human primate CD4, or fragment thereof which binds to HIV or SIV gp120, and

(b) detecting whether a complex has formed.

The invention is related to the discovery that non-human primates have CD4 of differing amino acid sequence than human CD4. The invention is also related to the discovery that when non-human primate CD4 is expressed on the surface of human cells, strikingly fewer multinucleated giant cells, or syncytia, are formed than when human CD4 is expressed on the surface of the cell. The invention is also related to the discovery that the presence of a glycine residue at position 87 in the non-human primate CD4 derived from the chimpanzee, instead of the glutamic acid residue as found in human CD4, is responsible for the lack of syncytia formation. As a result, the CD4 molecule derived from the chimpanzee can now be used in therapeutic application without the potential of causing syncytia formation.

The invention is also related to the unexpected discovery that chimpanzee CD4 contains two glycosylation sites (positions 32 and 66 (ASN)). This discovery allows for the preparation of glycosylated gp120 binding molecules and fragments thereof which bind to gp120 and likely have enhanced stability in vivo . Advantageously, the glycosylated gp120 binding molecules and fragments thereof may be administered less frequently to an animal than human or other primate CD4 molecules which are not glycosylated. Thus, the invention also relates to primate (including human) CD4 molecules having one or more glycosylation sites, for example, the chimp sequence at amino acid reidues 34 and 68, at 34 only, and at 68 only. The invention also relates to other CD4 molecules with glycosylation sites at different positions, so long as the molecule retains binding to gp120.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

THe invention is directed to nucleic acid molecules specifying non-human primate CD4, HIV gp120 binding fragments thereof, HIV gp120 binding soluble fragments thereof, fusion proteins thereof, and immunoglobulin-like molecules. The invention also relates to gp120 binding molecules capable of being glycosylated, HIV gp120 binding fragments thereof, fusion proteins thereof, and immunoglobulin-like molecules thereof. The nucleic acid molecules of the invention may be a DNA or RNA molecule.

By the term "soluble" is intended that the CD4 fragment is soluble in aqueous solutions which include, but are not limited to, detergent-free aqueous buffers and body fluids such as blood, plasma and serum.

The invention is also directed to the expression of these novel nucleic acid molecules in transformed hosts to give proteins and glycoproteins. The invention also relates to the use of these proteins and glycoproteins to treat and diagnose HIV infections.

In particular, the invention relates to expressing said nucleic acid molecules, which specify a fusion protein comprising an immunoglobulin light or heavy chain, in mammalian hosts which express complementary light or heavy chain immunoglobulins to give an immunoglobulin-like molecule which binds to HIV or SIV gp120.

The CD4 proteins, glycoproteins, CD4 fragments, gp120 binding molecules, fusion proteins and immunoglobulin-like molecules of the invention may be administered to an animal for the purpose of treating HIV or SIV infections. By the terms "HIV infections" is intended the condition of having AIDS, AIDS related complex (ARC) or where an animal harbors the AIDS virus, but does not exhibit the clinical symptoms of AIDS or ARC. By the terms "SIV infections" is intended the condition of being infected with simian immunodeficiency virus.

By the term "animal" is intended all animals which may derive benefit from the administration of the

CD4 proteins, glycoproteins, CD4 fragments, gp120 binding molecules, fusion proteins and Immunoglobulin-like molecules of the invention. foremost among such animals are humans, however, the invention is not intended to be so limited.

By the term "fusion protein" is intended a fused protein comprising a CD4 molecule of the invention, or fragment thereof which is capable of binding to gp120, linked at its C-terminus to an immunoglobulin chain wherein a portion of the N-terminus of the immunoglobulin is replaced with non-human primate CD4. Alternatively, the CD4 molecule or fragment thereof may be linked to a cytotoxic polypeptide such as ricin or diphtheria toxin.

By the term "non-human primate" is intended any member of the suborder Anthropoidea except for the family Hominidae. Such non-human primates include the superfamily Ceboidea, family Cebidae (the New World monkeys including the capuchins, howlers, spider monkeys and squirrel monkeys) and family Callithricidae (including the marmosets); the superfamily Cercopithecoidea, family Cercopithecidae (including the macaques, mandrills, baboons, proboscis monkeys, mona monkeys, and the sacred hanuman monkeys of India); and superfamily Hominoidae, family Pongidae (including gibbons, orangutans, gorillas, and chimpanzees). The rhesus monkey is one member of the macaques.

The nucleic acid molecules and proteins of the invention may be prepared according to the methods disclosed herein and according to well known methods of solid phase synthesis using the amino acid and DNA sequences disclosed herein.

As described more fully in the examples below, the gly residue at position 87 of the CD4 derived from the chimpanzee differs from the Glu residue present in human CD4 which is responsible for syncytium formation. This discovery allows for the preparation of new CD4 molecules which do not mediate syncytium formation. An example of such a protein related to the chimpanzee CD4 molecule comprises the following amino acid sequence:

MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGln-@-Lys
IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArg-#-
AspSerArgArgSerLeuTrpAspGlnGlyAsnPhe-$-LeuIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu

LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGlnGlyGlnSerLeuThr
LeuThrLeuGluSerProProGlySerSerProSerValGlnCysArgSerProArgGly
LysAsnIleGlnGlyGlyLysThrLeuSerValSerGlnLeuGluLeuGlnAspSerGly
ThrTrpThrCysThrValLeuGlnAsnGlnLysLysValGluPheLysIleAspIleVal
ValLeuAlaPheGlnLysAlaSerSerIleValTyrLysLysGluGlyGluGlnValGlu
PheSerPheProLeuAlaPheThrValGluLysLeuThrGlySerGlyGluLeuTrpTrp
GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu
ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu
HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla
LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr
GlnLeuGlnLysAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuMetLeu
SerLeuLysLeuGluAsnLysGluAlaLysValSerLysArgGluLysAlaValTrpVal
LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu
GluSerAsnIleLysValLeuProThrTrpSerThrProValGlnProMetAlaLeuIle
ValLeuGlyGlyValAlaGlyLeuLeuLeuPheIleGlyLeuGlyIlePhePheCysVal
ArgCysArgHisArgArgArgGlnAla-%-ArgMetSerGlnIleLysArgLeuLeuSer
GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIle,

wherein
-@- is Thr or Ile,
-#- is Val or Ala,
-$- is Thr or Pro, and
-%- is Gln or Glu,
or the glycosylated derivative thereof.

The recombinant DNA molecules which encode this family of proteins and glycoproteins have the following sequence:

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  1  ATGAACCGGGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCACTCCTCCCA
     GCAGCCACTCAGGGAAAGAAAGTGGTGCTGGGCAAGAAAGGGGACACAGTGGAACTGACC
121  TGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGAYAAAG
     ATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGYT
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 241  GACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTTWCCCTGATCATCAAGAATCTTAAG
      ATAGAAGACTCAGATACTTACATCTGTGAAGTGGGGGACCAGAAGGAGGAGGTGCAATTG
 361  CTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACC
      CTGACCTTGGAGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGT
 481  AAAAACATACAGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGC
      ACCTGGACATGCACTGTCTTGCAGAACCAGAAGAAAGTGGAGTTCAAAATAGACATCGTG
 601  GTGCTAGCTTTCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGGAG
      TTCTCCTTCCCACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGG
 721  CAGGCGGAGAGGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAA
      GTGTCTGTAAAACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTC
 841  CACCTCACCCTGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACCCTGGCC
      CTTGAAGCGAAAACAGGAAAGTTGCATCAGGAAGTGAACCTCGTGGTGATGAGAGCCACT
 961  CAGCTCCAGAAAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTG
      AGCTTGAAACTGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGGCGGTGTGGGTG
1081  CTGAACCCTGAGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTG
      GAATCCAACATCAAGGTTCTGCCCACATGGTCCACCCCGGTGCAGCCAATGGCCCTGATT
1201  GTGCTGGGGGGCGTCGCCGGCCTCCTGCTTTTCATTGGGCTAGGCATCTTCTTCTGTGTC
      AGGTGCCGGCACCGAAGGCGCCAAGCASAGCGGATGTCTCAGATCAAGAGACTCCTCAGT
1321  GAGAAGAAGACCTGCCAGTGCCCTCACCGGTTTCAGAAGACATGTAGCCCCATTTGA
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wherein Y is C or T,
W is A or C, and
S is C or G;
or a degenerate variant thereof.

In general, for the preparation of fusion proteins comprising an immunoglobulin, that portion of immunoglobulin which is deleted is the variable region. The fusion proteins of the invention may also comprise immunoglobulins where more than just the variable region has been deleted and replaced with the CD4 molecule or HIV gp120 binding fragment thereof. for example, the V_H and CH1 regions of an immunoglobulin chain may be deleted. In practice, any amount of the H-terminus of the immunoglobulin heavy chain can be deleted as long as the remaining fragment mediates cell death by antibody effector function or other mechanism. The minimum sequence required for binding complement encompasses domains CH2 and CH3. Joining of Fc portions by the hinge region is advantageous for increasing the efficiency of complement binding.

The CD4 molecules of the invention and fusion proteins thereof may comprise the complete CD4 sequence, the 372 amino acid extracellular region and the membrane spanning domain, or just the extracellular region. Moreover, the fusion proteins may comprise fragments of the extracellular region which retains binding to HIV gp120. The extracellular domain of CD4 consists of four contiguous regions each having amino acid and structural similarity to the variable and joining (V-J) domains of immunoglobulin light chains as well as related regions in other members of the immunoglobulin gene superfamily. These

structurally similar regions of CD4 are termed the V_1, V_2, V_3 and V_4 domains. See PCT Application Publication Number WO 89/02922 (published October 3, 1988). Thus, the non-human primate CD4 and fusion proteins thereof may comprise any combination of such binding regions. In general, any fragment of the CD4 proteins and glycoproteins of the invention may be used as long as they retain binding to gp120.

Gp120 binding CD4 fragments may be obtained by cutting the DNA sequence which encodes chimpanzee CD4 at the Nhe site at position 603 (to give a molecule which encodes two binding domains) or the BspM1 site at position 405 (to give a molecule which encodes one domain). Alternatively, the DNA molecule encoding rhesus CD4 may be cut at the Nhe site at position 603 (to give a molecule which encodes two domains) or the BspM1 site at position 405 (to give a molecule which encodes one domain). Other fragments may be obtained using, for example, an exonuclease. The DNA fragment can then be incorporated into a cloning vector and introduced into a host, followed by screening the transformed host for the presence of a protein which binds gp120. Methods for screening clones for specific binding activity are well known to those of ordinary skill in the art. Preferably, such CD4 fragments are soluble in aqueous solution.

Where the fusion protein comprises an immunoglobulin light chain, it is necessary that no more of the Ig chain be deleted than is necessary to form a stable complex with a heavy chain Ig. In particular, the cysteine residues necessary for disulfide bond formation must be preserved on both the heavy and light chain moieties.

When expressed in a host, e.g., a mammalian cell, the fusion protein may associate with other light or heavy Ig chains secreted by the cell to give a functioning immunoglobulin-like molecule which is capable of binding to gp120. The gp120 may be in solution, expressed on the surface of infected cells, or may be present on the surface of the HIV virus itself. Alternatively, the fusion protein may be expressed in a mammalian cell which does not secrete other light or heavy Ig chains. When expressed under these conditions, the fusion protein may form a homodimer.

Genomic or cDNA sequences may be used in the practice of the invention. Genomic sequences are expressed efficiently in myeloma cells, since they contain native promoter structures.

The constant regions of the antibody cloned and used in the chimeric immunoglobulin-like molecule may be derived from any mammalian source. They may be complement binding or ADCC active. The constant regions may be derived from any appropriate isotype, including IgG1, IgG3, or IgM.

The joining of various DNA fragments, is performed in accordance with conventional techniques, employing blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. The genetic construct may optionally encode a leader sequence to allow efficient expression of the fusion protein. For example, the leader sequence utilized by Maddon et al., Cell 42 :93-104 (1985) for the expression of human CD4 may be used.

For cDNA isolation, cDNA libraries may be screened, for example, by use of a complementary probe or by assay for the expressed CD4 molecule of the invention using a CD4-specific antibody. Methods for preparing antibodies by immunizing animals with an antigen are taught, for example, by Kohler and Milstein, Nature (London) 256 :495 (1975); Kohler et al ., Eur. J. Immunol. 6 :511 (1976); Kohler et al. , Eur. J. Immunol. 6 :292 (1976); or Hammerling et al ., in: Monoclonal Antibodies and T-Cell Hybridomas , Elsevier, N.V., pp.563-681 (1981). The invention further relates to monoclonal and polyclonal antibodies which are specific for the non-human CD4 proteins, glycoproteins of the invention, and the soluble and non-soluble fragments thereof.

The non-human primate CD4 may be derived from any member of the suborder Anthropoidea except for the family Hominidae. Preferably, the non-human primate CD4 is derived from the rhesus monkey or chimpanzee, although the invention is not intended to be so limited. One of ordinary skill in the art can obtain tee CD4 from any additional primate by isolation of the poly-A containing RNA of mitogen stimulated peripheral blood mononuclear cells obtained from the particular animal. After preparation of cDNA with, for example, reverse transcriptase, the cDNA may be ligated into an appropriate cloning vector and used to transform an appropriate host. The clones may then be screened with a monoclonal antibody directed to the rhesus monkey or chimpanzee CD4 of the invention followed by selection of positive clones, or by hybridization with the chimp or rhesus CD4 cDNAs.

To express the CD4 molecules and fusion hybrid proteins of the invention, transcriptional and translational signals recognized by an appropriate host element are necessary. Eukaryotic hosts which may be used include mammalian cells capable of culture in vitro , particularly leukocytes, more particularly myeloma cells or other transformed or oncogenic lymphocytes, e.g., EBV-transformed cells. Advantageously, mammalian cells are used to express the glycosylated CD4 proteins. Alternatively, non-mammalian cells may be employed, such as bacteria, fungi, e.g., yeast, filamentous fungi, or the like.

Preferred hosts for fusion protein production are mammalian cells, grown in vitro in tissue culture or in vivo in animals. Mammalian cells provide post translational modification to immunoglobulin protein molecules which provide for correct folding and glycosylation of appropriate sites. Mammalian cells which may be useful as hosts include cells of fibroblast origins such as VERO or CHO-K1 or cells of lymphoid origin, such as the hybridoma SP2/0-AG14 or the myeloma P3x63Sgh, and their derivatives. For the purpose of preparing an immunoglobulin-like molecule, a plasmid containing a gene which encodes a heavy chain immunoglobulin, wherein the variable region has been replaced with one of the CD4 molecules of the invention, may be introduced, for example, into J558L myeloma cells, a mouse plasmacytoma expressing the lambda-1 light chain but which does not express a heavy chain (see Oi et al. , P.N.A.S. (USA) 80 :825-829 (1983)). Other preferred hosts include COS cells, BHK cells and hepatoma cells.

The constructs may be joined together to form a single DNA segment or may be maintained as separate segments, by themselves or in conjunction with vectors.

Where the protein is not glycosylated, any host may be used to express the protein which is compatible with replication and transcription of sequences in the expression plasmid. In general, vectors containing replication and transcription controlling sequences are derived from species compatible with a host cell are used in connection with the host. The vector ordinarily carries a replication origin, as well as specific genes which are capable of providing phenotypic selection in transformed cells. The expression of the non-human primate CD4 molecules and fusion proteins can also be placed under control with other regulatory sequences which may be homologous to the organism in its untransformed state. For example, lactose-dependent E. coli chromosomal DNA comprises a lactose or lac operon which mediates lactose utilization by elaborating the enzyme beta-galactosidase. The lac control elements may be obtained from bacterial phage lambda placs, which is infective for E. coli . The lac promoter-operator system can be induced by IPTG.

Other promoters/operator systems or portions thereof can be employed as well. For example, colicin E1, galactose, alkaline phosphatase, tryptophan, xylose, tax , and the like can be used.

For mammalian hosts, several possible vector systems are available for expression. One class of vectors utilize DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or MOMLV), or SV40 virus. Cells which have stably integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow selection of transfected host cells. The marker may provide for prototropy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals such as copper or the like. The selectable marker gene can be either directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include splice signals, as well as transcriptional promoters, enhancers, and termination signals. The cDNA expression vectors incorporating such elements includes those described by Okayama, H., Mol. Cel. Biol. , 3 :280 (1983) and others.

Once the vector or DNA sequence containing the constructs has been prepared for expression, the DNA constructs may be introduced to an appropriate host. Various techniques may be employed, such as protoplast fusion, calcium phosphate precipitation, electroporation or other conventional techniques. After the fusion, the cells are grown in media and screened for the appropriate activity. Expression of the gene(s) results in production of the desired protein. If the expressed product is a fusion protein, it may then be subject to further assembly with an immunoglobulin light or heavy chain to form an immunoglobulin-like molecule.

The host cells for CD4 protein and glycoprotein, CD4 fragment, and immunoglobulin production may be immortalized cells, primarily myeloma or lymphoma cells. These cells may be grown in appropriate nutrient medium in culture flasks or injected into a synergistic host, e.g., mouse or a rat, or immunodeficient host or host site, e.g., nude mouse or hamster pouch. In particular, the cells may be introduced into the abdominal cavity of an animal to allow production of ascites fluid which contains the immunoglobulin-like. molecule. Alternatively, the cells may be injected subcutaneously and the chimeric antibody is harvested from the blood of the host. The cells may be used in the same manner as hybridoma cells. See Diamond et al. , N. Eng. J. Med. 304 :1344 (1981), and Kennatt, McKearn and Bechtol (Eds.), Monoclonal Antibodies: Hybridomas: – A New Dimension in Biologic Analysis , Plenum, 1980.

The CD4 proteins, glycoproteins, CD4 fragments, fusion proteins and immunoglobulin-like molecules of the invention may be isolated and purified in accordance with conventional conditions, such as extraction, precipitation, chromatography, affinity chromatography, electrophoresis or the like. For example, the CD4 proteins, glycoproteins and fragments may be purified by passing a solution thereof through a column having gp120 immobilized thereon (see U.S. patent No. 4,725,669). The bound CD4 molecule may then be eluted by treatment with a chaotropic salt or by elution with aqueous acetic acid (1 M).

The Ig fusion proteins may be purified by passing a solution containing the fusion protein through a column which contains immobilized protein A or protein G which selectively binds the Fc portion of the fusion protein. See, for example, Reis, K.J., et al. , J. Immunol. 132 :3098-3102 (1984); PCT Application, Publication No. W087/00329. The chimeric antibody may the be eluted by treatment with a chaotropic salt or by elution with aqueous acetic acid (1 M).

Alternatively the non-human primate CD4 proteins and glycoproteins, fragments, fusion proteins and immunoglobulin-like molecules may be purified on anti-CD4 antibody columns, or on anti-immunoglobulin antibody columns to give a substantially pure protein.

By the term "substantially pure" is intended that the protein is free of the impurities that are naturally associated therewith. Substantial purity may be evidenced by a single band by electrophoresis.

In one embodiment of the invention, cDNA sequences which encode the CD4 molecules of the invention, or a fragment thereof which binds gp120, may be ligated into an expression plasmid which codes for an antibody wherein the variable region of the gene has been deleted. Methods for the preparation of genes which encode the heavy or light chain constant regions of immunoglobulins are taught, for example, by Robinson, R. et al. , PCT Application, Publication No. WO87-02671. The cDNA sequence encoding the CD4 molecule or fragment may be directly joined to the cDNA encoding the light or heavy Ig contant regions or may be joined via a linker sequence. Preferably, the linker sequence does not encode a protein product which gives rise to an antigenic reaction in the individual.

Preferred immunoglobulin-like molecules which contain the CD4 molecules of the invention, or fragments thereof, contain the constant region of an IgM, IgG1 or IgG3 antibody.

The CD4 proteins, glycoproteins, fragments, fusion proteins and immunoglobulin-like molecules, and pharmaceutical compositions thereof may be used for the treatment or prophylaxis of HIV viral infections. This method comprises administering to an animal an effective amount of the CD4 proteins, glycoproteins, fragments, fusion proteins and immunoglobulin-like molecules, and pharmaceutical compositions thereof, which are capable of specifically forming a complex with gp120 so as to render the HIV or SIV, with which the individual is infected, incapable of infecting $T4^+$ cells.

The fusion protein and immunoglobulin-like molecule may complex to gp120 which is expressed on infected cells. Although the inventor is not bound by a particular theory, it appears that the Fc portion of the fusion protein or immunoglobulin-like molecule may bind with complement to mediate destruction of the cell. In this manner, infected cells are destroyed so that additional viral particle production is stopped.

For the purpose of treating HIV infections, the non-human primate CD4 molecules or fragments thereof, fusion proteins or immunoglobulin-like molecules of the invention may additionally contain a radiolabel, therapeutic agent or cytotoxic polypeptide which enhances destruction of the HIV particle or HIV-infected cell.

Examples of radioisotopes which can be bound to the proteins, glycoproteins, fusion proteins, and immunoglobulin-like molecules of the invention for use in HIV-therapy are ^{125}I, ^{131}I, ^{90}Y, ^{67}Cu, ^{217}Bi, ^{211}At, ^{212}Pb, ^{47}Sc, and ^{109}Pd. Optionally, a label such as boron can be used which emits a and β particles upon bombardment with neutron radiation.

For in vivo diagnosis radionucleotides may be bound to the CD4 proteins, glycoproteins or fragments thereof, fusion proteins or immunoglobulin-like molecules either directly or by using an intermediary functional group. An intermediary group which is often used to bind radioisotopes, which exist as metallic cations, to antibodies is diethylenetriaminepentaacetic acid (DTPA). Typical examples of metallic cations which are bound in this manner are ^{99m}Tc ^{123}I, ^{111}In, ^{131}I, ^{97}Ru, ^{67}Cu, ^{67}Ga, and $^{6^*}Ga$.

Moreover, the CD4 proteins and glycoproteins or fragments thereof, fusion proteins and immunoglobulin-like molecules may be tagged with an NMR imaging agent which include paramagnetic atoms. The use of an NMR imaging agent allows the in vivo diagnosis of the presence of and the extent of HIV infection within a patient usingNMR techniques. Elements which are particularly useful in this manner are ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr, and ^{56}Fe.

Introduction of the nucleic acid molecules of the invention by gene therapy may also be contemplated, for example, using retroviruses or other means to introduce the genetic material specifying the fusion proteins into suitable target tissues. In this embodiment, the target tissues having the nucleic acid molecules of the invention may then produce the CD4 molecules or fusion protein in vivo .

The nucleic acid molecules specifying the CD4 molecules or fragments thereof may be used to reconstitute the immune system of an individual suffering from HIV. For example, the bone marrow cells of an HIV-infected individual may be removed and the hematopoietic stem cells, either as part of a mixed population or a purified fraction, may be infected or transfected with a virus or DNA construct that specifies the non-human primate CD4 or fragment thereof. Production of human CD4 may be shut down by including within the same or different genetic construct, a gene which interferes with the expression of human CD4.

Such a gene may take many forms, for example, it may encode RNA that binds to a regulatory protein (since the non-human primate CD4 may be under other control, its expression will not be affected); an antisense RNA that binds selectively to the human CD4 gene; or a DNA-binding protein that has had its regulatory region amputated. The modified stem cells would then be injected back into the patient where they will migrate to the bone marrow. Preferably, the marrow would have been previously cleared of normal hematopoietic cells by irradiation or with a toxic drug. See Baltimore, D. Nature 335 :395-396 (1988).

Methods for the transfection of hematopoietic cells are well known and taught, for example, by Wetherall, D.J., Nature 331 :13-14 (1988); Dick, J.E., Ann. N. Y. Acad. Sci. 507 :242-251 (1987); Eglitis, D.B. et al ., Science 230 :1395-1398 (1985); Gillio, A. et al. , Ann. N.Y. Acad. Sci. 511 :406-417 (1987). Methods for the transfection of cells with anti-sense RNA are taught, for example, by Hambor, J.E. et al ., Proc. Natl. Acad. Sci. (USA) 85 :4010-4014 (1988); Sanford, J.C., J. Theor. Biol. 130 :469-480 (1988); Izant, J.G. et al. , Science 229 :345-352 (1985); and Hambor, J.E. et al. , J. Exa. Med. 168 :1237-1245 (1988).

The non-human primate CD4, and soluble and non-soluble fragments thereof which bind HIV or SIV gp120, may also be used in vivo to treat HIV infection by blocking infection of human CD4 bearing lymphocytes and syncytium formation. See Lui, M. et al ., J. Clin. Invest.82 :2176-2180 (1988) or Fischer, R.A. et al. , Nature 331 :76-78 (1988) for a discussion on the use of human CD4 and soluble fragments thereof to block HIV infection of CD4 bearing lymphocytes and syncytium formation.

Fusion proteins comprising the CD4 proteins, glycoproteins and fragments thereof, and a therapeutic agent can also be used to treat HIV infected individuals by killing HIV-infected cells in vivo . Therapeutic agents may include, for example, cytotoxic polypeptides such as the bacterial toxins diphtheria toxin or ricin. Methods for producing fusion proteins comprising fragment A of diphtheria toxin are taught in U.S. Patent 4,675,382 (1987) which is incorporated by reference herein. Diphtheria toxin contains two polypeptide chains. The B chain binds the toxin to a receptor on a cell surface. The A chain actually enters the cytoplasm and inhibits protein synthesis by inactivating elongation factor 2, the factor that translocates ribosomes along mRNA concomitant with hydrolysis of ATP. See Darnell, J., et al ., in Molecular Cell Biology , Scientific American Books, Inc., page 662 (1986). Alternatively, a fusion protein comprising ricin, a toxic lectin, may be prepared. Methods for the preparation of a fusion protein comprising human CD4 linked to active regions of Pseudomonas endotoxin A and the use thereof to selectively kill HIV infected cells are taught by Chaudhary, V.K. et al. , Nature 335 :369-372 (1988), which is incorporated by reference herein.

The dose ranges for the administration of the CD4 proteins, glycoproteins and fragments thereof, fusion proteins and immunoglobulin-like molecules are those which are large enough to produce the desired effect whereby the symptoms of HIV or SIV infection are ameliorated. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counter indications, if any, immune tolerance and other such variables, to be adjusted by the individual physician. Dosage can vary from .001 mg/kg to 50 mg/kg, preferably 0.1 mg/kg to 1.0 mg/kg, of the CD4 molecule of the invention, gp120 binding molecule, or fragment thereof, fusion protein, or immunoglobulin-like molecule, in one or more administrations daily, for one or several days. The immunoglobulin-like molecule can be administered parenterally by injection or by gradual perfusion over time. They can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

Preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, anti-oxidants, chelating agents, inert gases and the like. See, generally, Remington's Pharmaceutical Science , 16th Ed., Mack Eds., 1980.

The invention also relates to a method for preparing a medicament or pharmaceutical composition comprising the components of the invention, the medicament being used for therapy of HIV or SIV infection in animals.

The proteins and glycoproteins of the present invention may also be used in combination with other therapeutics used in the treatment of AIDS, ARC and HIV infection. For example, the proteins and glycoproteins may be co-administered with anti-retroviral agents that block reverse transcriptase such as AZT, DDI, HPA-23, phosphonoformate, suramin, ribavirin and deoxycytidine. Alternatively, the proteins and glycoproteins of the invention may be co-administered with such anti-viral agents as interferons, including alpha interferon, gamma interferon, omega interferon, or glucosidase inhibitors such as castanospermine.

Such combination therapies may advantageously utilize lower dosages of those agents so as to minimize toxicity and enhance the effectiveness of the treatment.

The detection and quantitation of antigenic substances and biological samples frequently utilizes immunoassay techniques. These techniques are based upon the formation of the complex between the antigenic substance, e.g., gp120, being assayed and an antibody or antibodies in which one or the other member of the complex may be detectably labeled. In the present invention, the CD4 proteins, glycoproteins or fragments thereof, immunoglobulin-like molecules or fusion proteins may be labeled with any conventional label.

Thus, the CD4 protein, glycoprotein or fragment thereof, fusion protein or immunoglobulin-like molecule can also be used in assay for HIV or SIV viral infection in a biological sample by contacting a sample, derived from an animal suspected of having an HIV or SIV infection, with the CD4 protein, glycoprotein or fragment thereof, fusion protein or immunoglobulin-like molecule, and detecting whether a complex with gp120, either alone or on the surface of an HIV-infected cell, has formed.

For example, a biological sample may be treated with nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble protein. The support may then be washed with suitable buffers followed by treatment with the CD4 protein, glycoprotein or fragment thereof, fusion protein, or immunoglobulin-like molecule, any of which may be detectably labeled. The solid phase support may then be washed with a buffer a second time to remove unbound protein and the label detected.

In carrying out the assay of the present invention on a sample containing gp120, the process comprises:

a) contacting a sample suspected of containing gp120 with a solid support to effect immobilization of gp120, or cell which expresses gp120 on its surface;

b) contacting said solid support with the detectably labeled CD4 protein, glycoprotein or fragment thereof which binds to HIV gp120, immunoglobulin-like molecule or fusion protein molecule of the invention;

c) incubating said detectably labeled molecule with said support for a sufficient amount of time to allow the detectably labelled molecule to bind to the immobilized gp120 or cell which expresses gp120 on its surface;

d) separating the solid phase support from the incubation mixture obtained in step c); and

e) detecting the bound detectably labeled molecule and thereby detecting and quantifying gp120.

Alternatively, the detectably labeled CD4 protein, glycoprotein or fragment thereof, immunoglobulin-like molecule or fusion protein - gp120 complex in a sample may be separated from a reaction mixture by contacting the complex with an immobilized antibody or protein which is specific for an immunoglobulin or, e.g., protein A, protein G, anti-IgM or anti-IgG antibodies. Such anti-immunoglobulin antibodies may be monoclonal or polyclonal. The solid support may then be washed with suitable buffers to give an immobilized complex. The label may then be detected to give a measure of gp120 and, thereby, the presence of HIV.

This aspect of the invention relates to a method for detecting HIV or SIV viral infection in a sample comprising: (a) contacting a sample suspected of containing gp120 with a fusion protein comprising non-human primate CD4 or fragment thereof that binds to HIV gp120 and the Fc portion of an immunoglobulin chain, and

(b) detecting whether a complex is formed.

The invention also relates to a method of detecting gp120 in a sample, further comprising:

(c) contacting the mixture obtained in step (a) with an Fc binding molecule, such as an antibody, protein A, or protein G, which is immobilized on a solid phase support and is specific for the fusion protein, to give a gp120 fusion protein-immobilized antibody complex

(d) washing the solid phase support obtained in step (c) to remove unbound fusion protein, and

(e) and detecting the label on the fusion protein.

Of course, the specific concentrations of detectably labeled immunoglobulin-like molecule (or fusion protein) and gp120, the temperature and time of incubation, as well as other assay conditions may be varied, depending on various factors including the concentration of gp120 in the sample, the nature of the sample, and the like. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

Other such steps as washing, stirring, shaking, filtering and the like may be added to the assays as is necessary for the particular situation.

One of the ways in which the CD4 protein, glycoprotein or fragment thereof, immunoglobulin-like molecule or fusion protein can be detectably labeled is by linking the same to an enzyme. This enzyme, in turn, when later exposed to its substrate, will catalize the formation of a product which can be detected as, for example, by spectrophotometric, fluorometric or by visual means. Enzymes which can be used to

detectably label the CD4 protein, glycoprotein or fragment thereof, immunoglobulin-like molecule or fusion protein of the present invention include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-VI-phosphate dehydrogenase, glucoamylase and acetylcholine esterase.

The CD4 protein, glycoprotein or fragment thereof, immunoglobulin-like molecule or fusion protein of the present invention may also be labeled with a radioactive isotope which can be determined by such means as the use of a gamma counter or a scintillation counter or by autoradiography. Isotopes which are particularly useful for the purpose of the present invention are: ^{3}H, ^{125}I, ^{131}I, ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{36}Cl, ^{57}Co, ^{58}Co, ^{59}Fe and ^{75}Se.

It is also possible to label the CD4 protein, glycoprotein or fragment thereof, immunoglobulin-like molecule or fusion protein with a fluorescent compound. When the fluorescently labeled immunoglobulin-like molecule is exposed to light of the proper wave length, its presence can then be detected due to the fluorescence of the dye. Among the most commonly used fluorescent labelling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o -phthaldehyde and fluorescamine.

The CD4 protein, glycoprotein or fragment thereof, immunoglobulin-like molecule or fusion protein of the invention can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the CD4 protein, glycoprotein or fragment thereof, immunoglobulin-like molecule or fusion protein, using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

The CD4 protein, glycoprotein or fragment thereof, immunoglobulin-like molecule or fusion protein of the present invention also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged CD4 protein, glycoprotein or fragment thereof, immunoglobulin-like molecule or fusion protein is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used to label the CD4 protein, glycoprotein or fragment thereof, immunoglobulin-like molecule or fusion protein of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important biolum inescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

Detection of the CD4 protein, glycoprotein or fragment thereof, immunoglobulin-like molecule or fusion protein may be accomplished by a scintillation counter, for example, if the detectable label is a radioactive gamma emitter, or by a fluorometer, for example, if the label is a fluorescent material. In the case of an enzyme label, the detection can be accomplished by colorimetric methods which employ a substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

The assay of the present invention is ideally suited for the preparation of a kit. Such a kit may comprise a carrier means being compartmentalized to receive in close confinement therewith one or more container means such as vials, tubes and the like, each of said container means comprising the separate elements of the immunoassay. For example, there may be a container means containing a solid phase support, and further container means containing the detectably labeled CD4 protein, glycoprotein or fragment thereof, immunoglobulin-like molecule or fusion protein. Further container means may contain standard solutions comprising serial dilutions of analytes such as gp120 or fragments thereof to be detected. The standard solutions of these analytes may be used to prepare a standard curve with the concentration of gp120 plotted on the abscissa and the detection signal on the ordinate. The results obtained from a sample containing gp120 may be interpolated from such a plot to give the concentration of gp120.

The CD4 protein, glycoprotein or fragment thereof, immunoglobulin-like molecule or fusion protein of the present invention can also be used as a stain for tissue sections. For example, a labeled molecule comprising CD4 protein or glycoprotein or HIV gp120 binding fragment thereof, may be contacted with a tissue section, e.g., a brain biopsy specimen. This section may then be washed and the label detected.

The following examples are illustrative, but not limiting the method and composition of the present invention. Other suitable modifications and adaptations which are obvious to those skilled in the art are within the spirit and scope of this invention.

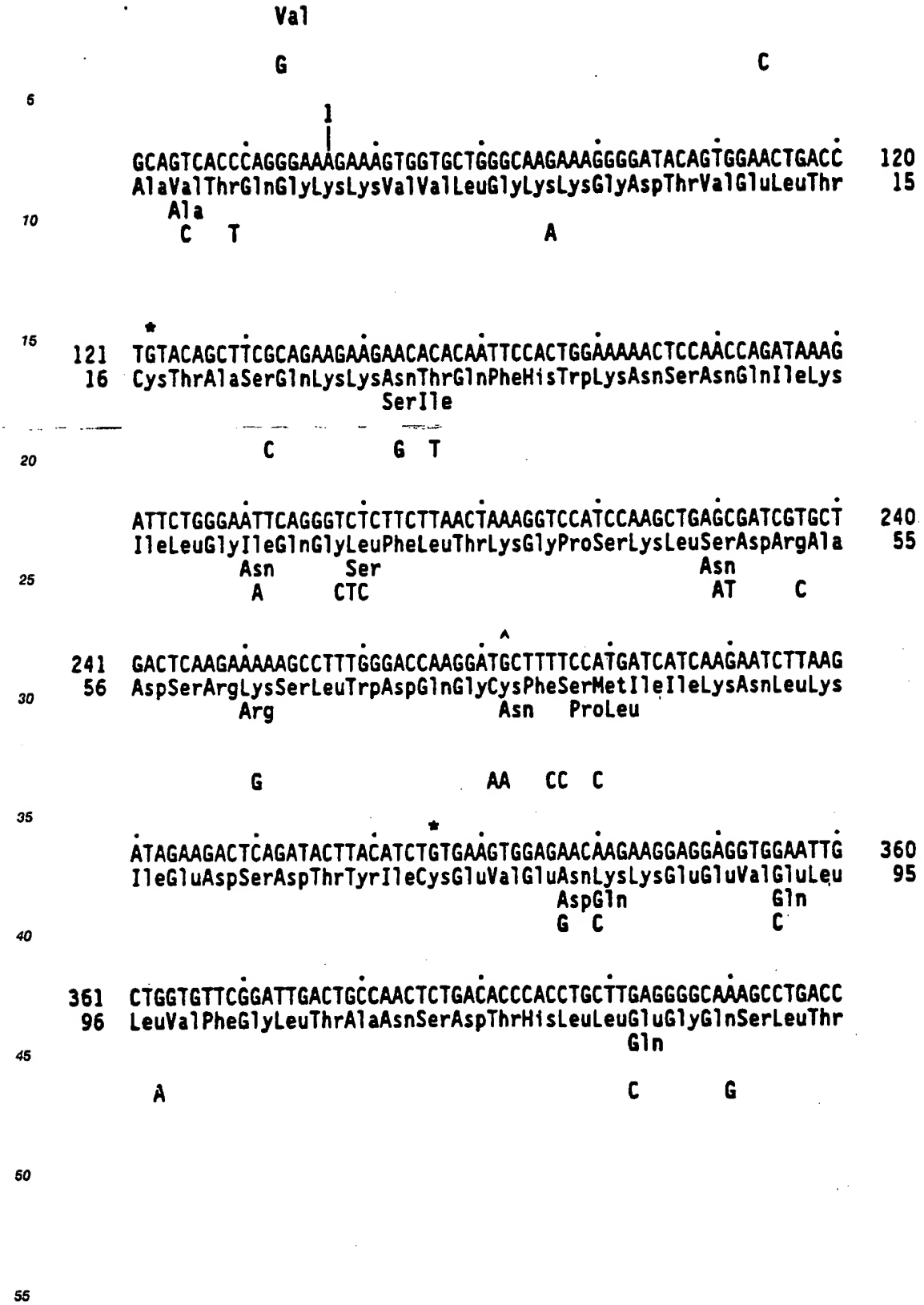
EXAMPLES

EXAMPLE 1 ISOLATION OF CHIMPANZEE AND RHESUS MONKEY CD4 cDNAs

cDNA clones encoding the CD4 antigens of the Chimpanzee (Pan troglodytes) and the Rhesus Monkey (Maccaca mulatta) were isolated, sequenced, and expressed. Non-human primate CD4 cDNAS were synthesized from the poly-A containing RNA of mitogen stimulated peripheral blood mononuclear cells obtained from these animals. cDNA expression libraries were made in the vector CDM8 and CD4 cDNAS we isolated by four rounds of immunoselection as previously described by Seed et al ., Proc. Natl. Acad. Sci (USA) 84 :3365-3369 (1987). Sequencing was carried out using the dideoxynucleotide chain termination technique on single and double stranded templates. The DNA and amino acid sequences of the Chimpanzee and Rhesus Monkey CD4 are shown below. Also shown is a comparison of the respective sequences to human CD4.

RHESUS CD4 CODING SEQUENCE AND PREDICTED AMINO ACID SEQUENCE SHOWING DIFFERENCES FROM HUMAN SEQUENCES

```
   1  ATGAACCGGGGAATCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTACTCCCA
-25  MetAsnArgGlyIleProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
```


Val
G
C
1
GCAGTCACCCAGGGAAAGAAAGTGGTGCTGGGCAAGAAAGGGGATACAGTGGAACTGACC 120
AlaValThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr 15
Ala
C T
A
121 TGTACAGCTTCGCAGAAGAAGAACACACAATTCCACTGGAAAAACTCCAACCAGATAAAG
16 CysThrAlaSerGlnLysLysAsnThrGlnPheHisTrpLysAsnSerAsnGlnIleLys
SerIle
C
G T
ATTCTGGGAATTCAGGGTCTCTTCTTAACTAAAGGTCCATCCAAGCTGAGCGATCGTGCT 240
IleLeuGlyIleGlnGlyLeuPheLeuThrLysGlyProSerLysLeuSerAspArgAla 55
Asn
Ser
Asn
A
CTC
AT
C
241 GACTCAAGAAAAAGCCTTTGGGACCAAGGATGCTTTTCCATGATCATCAAGAATCTTAAG
56 AspSerArgLysSerLeuTrpAspGlnGlyCysPheSerMetIleIleLysAsnLeuLys
Arg
Asn
ProLeu
G
AA
CC
C
ATAGAAGACTCAGATACTTACATCTGTGAAGTGGAGAACAAGAAGGAGGAGGTGGAATTG 360
IleGluAspSerAspThrTyrIleCysGluValGluAsnLysLysGluGluValGluLeu 95
AspGln
Gln
G C
C
361 CTGGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTGAGGGGCAAAGCCTGACC
96 LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGluGlyGlnSerLeuThr
Gln
A
C
G

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                                                    *
     CTGACCTTGGAGAGCCCCCCTGGTAGTAGCCCCTCAGTGAAATGTAGGAGTCCAGGGGGT   480
     LeuThrLeuGluSerProProGlySerSerProSerValLysCysArgSerProGlyGly   135
                                                  Gln           Arg
                                                  C             A

481  AAAAACATACAGGGGGGGAGGACCATCTCTGTGCCTCAGCTGGAGCGCCAGGATAGTGGC
136  LysAsnIleGlnGlyGlyArgThrIleSerValProGlnLeuGluArgGlnAspSerGly
                     Lys   Leu       Ser         Leu

                      A    C    C   T           T

          *
     ACCTGGACATGCACCGTCTCGCAGGACCAGAAGACGGTGGAGTTCAAAATAGACATCGTG   600
     ThrTrpThrCysThrValSerGlnAspGlnLysThrValGluPheLysIleAspIleVal   175
                     Leu   Asn       Lys
               T     T     A         A

601  GTGCTAGCTTTCCAGAAGGCCTCCAGCACAGTCTATAAGAAAGAGGGGGAACAGGTGGAG
176  ValLeuAlaPheGlnLysAlaSerSerThrValTyrLysLysGluGlyGluGlnValGlu
                                 Ile

                                  T

     TTCTCCTTCCCACTCGCCTTTACACTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGG   720
     PheSerPheProLeuAlaPheThrLeuGluLysLeuThrGlySerGlyGluLeuTrpTrp   215
                               Val
                               G

721  CAGGCGGAGAGGGCCTCCTCCTCCAAGTCTTGGATTACCTTCGACCTGAAGAACAAGGAA
216  GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu

               T                    C     T

     GTGTCTGTAAAACGGGTTACCCAGGACCCCAAGCTCCAGATGGGCAAGAAGCTCCCGCTC   840
     ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu   255
                                  T

                                                    ---------
841  CACCTCACCCTGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACGCTGGCC
256  HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla

                                                  C
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                CTTGAAGCGAAAACAGGAAAGTTGCATCAGGAAGTGAACCTCGTGGTGATGAGAGCCACT   960
                LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr   295

                                                    G

                          --------- *
           961  CAGTTCCAGGAAAATTTGACCTGTGAAGTGTGGGGACCCACCTCCCCTAAGCTGACGCTG
           296  GlnPheGlnGluAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuThrLeu
                   Leu   Lys                                              Met

                   C     A               G                          T

                AGCTTGAAACTGGAGAACAAGGGGGCAACGGTCTCGAAGCAGGCGAAGGCGGTGTGGGTG   1080
                SerLeuLysLeuGluAsnLysGlyAlaThrValSerLysGlnAlaLysAlaValTrpVal   335
                                    Glu   Lys         ArgGlu
                                     A     A          G  A

                                     *
          1081  CTGAACCCTGAGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTA
           336  LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu

                                                                       G

                GAATCCAACATCAAGGTTGTGCCCACATGGCCCACCCCGGTGCAGCCAATGGCCCTGATT   1200
                GluSerAsnIleLysValValProThrTrpProThrProValGlnProMetAlaLeuIle   375
                                    Leu         Ser                 ---------
                                    C           T

          1201  GTGCTGGGGGGCGTTGCGGGCCTCCTGCTTTTCACTGGGCTAGGCATCTTCTTCTGTGTC
           376  ValLeuGlyGlyValAlaGlyLeuLeuLeuPheThrGlyLeuGlyIlePhePheCysVal
                ---------------------------------Ile-----------------

                          C  C                T

                AGGTGCCGGCATCGAAGGCGTCAAGCAGAGCGGATGTCTCAGATCAAGAGACTCCTCAGT   1320
                ArgCysArgHisArgArgArgGlnAlaGluArgMetSerGlnIleLysArgLeuLeuSer   415

                          C        C

          1321  GAAAAGAAGACCTGCCAGTGCCCTCACCGGTTTCAGAAGACATGTAGCCCCATTTGA  1377
           416  GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIleEnd   433

                   G
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CHIMP CD4 CODING SEQUENCE AND PREDICTED AMINO ACID SEQUENCE SHOWING DIFFERENCES FROM HUMAN SEQUENCES

```
  1   ATGAACCGGGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCACTCCTCCCA
-25   MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro

                                                        G
                      1
                      |
      GCAGCCACTCAGGGAAAGAAAGTGGTGCTGGGCAAGAAAGGGGACACAGTGGAACTGACC  120
      AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr   15

                                         A        T

                                                      ^^^^^^^^^
       *
121   TGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGACAAAG
 16   CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnThrLys
                                                            Ile
                                                             T

      ATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGTT  240
      IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgVal   55
                                                               Ala
                                                                C

                                    ^^^^^^^^^
241   GACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTTACCCTGATCATCAAGAATCTTAAG
 56   AspSerArgArgSerLeuTrpAspGlnGlyAsnPheThrLeuIleIleLysAsnLeuLys
                                          Pro
                                         CC
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       ATAGAAGACTCAGATACTTACATCTGTGAAGTGGGGGACCAGAAGGAGGAGGTGCAATTG   360
       IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu   95
                               Glu
                                A

361    CTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACC
96     LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGlnGlyGlnSerLeuThr

       CTGACCTTGGAGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGT   360
       LeuThrLeuGluSerProProGlySerSerProSerValGlnCysArgSerProArgGly   135

481    AAAAACATACAGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGC
136    LysAsnIleGlnGlyGlyLysThrLeuSerValSerGlnLeuGluLeuGlnAspSerGly

       ACCTGGACATGCACTGTCTTGCAGAACCAGAAGAAAGTGGAGTTCAAAATAGACATCGTG   600
       ThrTrpThrCysThrValLeuGlnAsnGlnLysLysValGluPheLysIleAspIleVal   175

                                 G

601    GTGCTAGCTTTCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGGAG
176    ValLeuAlaPheGlnLysAlaSerSerIleValTyrLysLysGluGlyGluGlnValGlu

       TTCTCCTTCCCACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGG   720
       PheSerPheProLeuAlaPheThrValGluLysLeuThrGlySerGlyGluLeuTrpTrp   215

721    CAGGCGGAGAGGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAA
216    GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu

       GTGTCTGTAAAACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTC   840
       ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu   255

841    CACCTCACCCTGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACCCTGGCC
256    HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla

       CTTGAAGCGAAAACAGGAAAGTTGCATCAGGAAGTGAACCTCGTGGTGATGAGAGCCACT   840
       LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr   295

                                       G
```

```
                    ---------
961  CAGCTCCAGAAAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTG
296  GlnLeuGlnLysAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuMetLeu

     AGCTTGAAACTGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGGCGGTGTGGGTG  1080
     SerLeuLysLeuGluAsnLysGluAlaLysValSerLysArgGluLysAlaValTrpVal   335

1081 CTGAACCCTGAGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTG
 336 LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu

     GAATCCAACATCAAGGTTCTGCCCACATGGTCCACCCCGGTGCAGCCAATGGCCCTGATT  1200
     GluSerAsnIleLysValLeuProThrTrpSerThrProValGlnProMetAlaLeuIle   375
                                           ---------

1201 GTGCTGGGGGGCGTCGCCGGCCTCCTGCTTTTCATTGGGCTAGGCATCTTCTTCTGTGTC
 376 ValLeuGlyGlyValAlaGlyLeuLeuLeuPheIleGlyLeuGlyIlePhePheCysVal
     ----------------------------------------------------

     AGGTGCCGGCACCGAAGGCGCCAAGCACAGCGGATGTCTCAGATCAAGAGACTCCTCAGT  1320
     ArgCysArgHisArgArgArgGlnAlaGlnArgMetSerGlnIleLysArgLeuLeuSer   415
                                 Glu
                                 G

1321 GAGAAGAAGACCTGCCAGTGCCCTCACCGGTTTCAGAAGACATGTAGCCCCATTTGA  1377
 416 GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIleEnd   433
```

The chimpanzee CD4 antigen is 99% homologous to its human counterpart, possessing 5 amino acid substitutions in the 433 amino acid predicted mature polypeptide, while the rhesus monkey CD4 is 92% homologous having 34 divergences from the human CD4 amino acid sequence. Antigen expression was effected transiently in CDM8 as well as stably using the retroviral vector pMNCS.

EXAMPLE 2 CHARACTERIZATION OF THE HUMAN CD4 DOMAIN WHICH IS REQUIRED FOR HIV MEDIATED SYNCYTIUM FORMATION

These non-human primate CD4 antigens were expressed on human cells which were thereby rendered susceptible to infection by HIV, but formed strikingly fewer multinucleated giant cells, or syncytia, than their counterparts expressing the human CD4 antigen. Using In vitro mutagenesis this phenotype was localized to a single amino acid difference between the chimpanzee and human CD4 glycoproteins. This amino acid substitution quantitatively affects the ability of HeLa cells to form syncytia when these antigens are expressed in concert with the external and trans membrane proteins (EMP and TMP) of the human immunodeficiency virus type I (HIV). This was achieved by transiently expressing six trans-species hybrid CD4 antigens, which contain each of the three nonconservative extracellular amino acid sequence changes between the two species alone and in pairs, followed by infection with the Vaccinia:(HIV env) recombinant virus VSC25. The presence of a glycine residue at position 87, as found in chimpanzee CD4, instead of the glutamic acid residue found in human CD4, essentially eliminates the formation of multinucleated syncytia. Conversely the transfer of the human glutamic acid residue at position 87 to the chimpanzee CD4 confers the ability to form syncytia in the presence of HIV EMP and TMP. In contrast the absence or presence of

either of the two amino acid substitutions which create glycosylation sites unique to the chimpanzee CD4 antigen, at amino acids 34 and 68 in the first immunoglobulin variable region homologous domain, has little or no effect on the extent of syncytium formed in this assay. We expect that all of these hybrid CD4 glycoproteins will show equal affinity for HIV EMP, since none of these amino acid sequence differences are in the HIV binding site defined earlier.

If syncytium formation is an important mechanism of HIV induced disease this blockade of HIV mediated syncytium formation may account for the resistance of the chimpanzee to the pathology of the acquired immune deficiency syndrome (AIDS) despite prolonged infection by HIV.

EXAMPLE 3 PREPARATION OF CD4-IG cDNA CONSTRUCTS

The Extracellular portion of the chimpanzee or rhesus monkey coding sequence (encoding the signal peptides and amino acids 1-372 of the mature glycoproteins) is fused at three locations to a human IgG1 heavy chain constant region gene by means of a synthetic splice donor linker molecule. To exploit the splice donor linker, a BaMHI linker having the sequence CGCGGATCCGCG is first inserted at amino acid residue 395 of the CD4 precursor sequence (nucleotide residue 1295). A synthetic splice donor sequence

GATCCCGAGGGTGAGTACTA
GGCTCCCACTCATGATTCGA

bounded by BamHI and HindIII complementary ends is created and fused to the HindIII site in the intron preceding the CH1 domain, to the EspI site in the intron preceding the hinge domain, and to the BanI site preceding the CH2 domain of the IgG1 genomic sequence. Assembly of the chimeric genes by ligation at the BaMHI site affords molecules in which either the variable (V) region, the V+CH1 regions, or the V, CH1 and hinge regions are replaced by CD4. In the last case, the chimeric molecule is expected to form a monomer structure, while in the former, a dimeric molecule is expected.

Immunoprecipitation of the fusion proteins with a panel of monoclonal antibodies directed against CD4 epitopes will show that all of the epitopes are preserved. A specific high affinity association is demonstrated between the chimeric molecules and HIV envelope proteins expressed on the surface of cells transfected with an attenuated (reverse transcriptase deleted) proviral construct, or infected with a vaccinia:HIV env recombinant virus.

EXAMPLE 4 PREPARATION OF THE FUSION PROTEINS FROM SUPERNATANTS OF COS CELLS

COS cells grown in DME medium supplemented with 10% Calf Serum and gentamicin sulfate at 15 µg/ml are split into DME medium containing 10% NuSerum (Collaborative Research) and gentamicin to give 50% confluence the day before transfection. The next day, CsCl purified plasmid DNA is added to a final concentration of 0.1 to 2.0 µg/ml followed by DEAE Dextran to 400 µg/ml and chloroquine to 100 µM. After 4 hours at 37° C, the medium is aspirated and a 10% solution of dimethyl sulfoxide in phosphate buffered saline is added for 2 minutes, aspirated, and replaced with DME/10% Calf Serum. 8 to 24 hours later, the cells are trypsinized and split 1:2.

For radiolabeling, the medium is aspirated 40 to 48 hours after transfection, the cells are washed once with phosphate buffered saline, and DME medium lacking cysteine or methionine is added. 30 minutes later, ^{35}S-labeled cysteine and methionine are added to final concentrations of 30-60 µci and 100-200 µci respectively, and the cells allowed to incorporate label for 8 to 24 more hours. The supernatants are recovered and examined by electrophoresis on 7.5% polyacrylamide gels following denaturation and reduction, or on 5% polyacrylamide following denaturation without reduction. The IgG-CD4 fusion proteins form dimer structures. The CD4-IgM fusion proteins form large multimers beyond the resolution of the gel system without reduction, and monomers of the expected molecular mass with reduction.

Unlabeled proteins are prepared by allowing the cells to grow for 5 to 10 days post transfection in DME medium containing 5% NuSerum and gentamicin as above. The supernatants are harvested, centrifuged, and purified by batch adsorption to either protein A trisacryl, protein A agarose, goat anti-human IgG antibody agarose, rabbit anti-human IgM antibody agarose, or monoclonal anti-CD4 antibody agarose.

Antibody agarose conjugates are prepared by coupling purified antibodies to cyanogen bromide activated agarose according to the manufacturer's recommendations, and using an antibody concentration of 1 mg/ml. Following batch adsorption by shaking overnight on a rotary table, the beads are harvested by pouring into a sintered glass funnel and washed a few times on the funnel with phosphate buffered saline containing 1% Nonidet P40 detergent. The beads are removed from the funnel and poured into a small disposable plastic column (Quik-Sep QS-Q column, Isolab), washed with at least 20 column volumes of phosphate buffered saline containing 1% Nonidet P40, with 5 volumes of 0.15 M NaCl, 1 mM EDTA (pH 8.0), and eluted by the addition of either 0.1 M acetic acid, 0.1 M acetic acid containing 0.1 M NaCl, or 0.25 M glycine-HCl buffer, pH 2.5.

EXAMPLE 5 BLOCKAGE OF SYNCYTIUM FORMATION BY THE FUSION PROTEINS

Purified or partially purified fusion proteins are added to HPB-ALL cells infected 12 hours previously with a vaccinia virus recombinant encoding HIV envelope protein. After incubation for 6-8 more hours, the cells are washed with phosphate buffered saline, fixed with formaldehyde, and photographed. All of the full-length CD4 immunoglobulin fusion proteins will show inhibition of syncytium formation.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed with any wide range of equivalent parameters of composition, conditions, and methods of preparing such recombinant molecules, vectors, transformed hosts and proteins without departing from the spirit of scope of the invention or any embodiment thereof.

Claims

1. A nucleic acid molecule specifying non-human primate CD4, or an HIV gp120 binding fragment thereof, which preferably is soluble in aqueous solution.
2. The nucleic acid molecule of claim 1 which is DNA, RNA or is complementary to the nucleic acid molecule of claim 1.
3. The nucleic acid molecule of claim 1 which is detectably labeled.
4. The nucleic acid molecule of claim 1, wherein said non-human primate is the rhesus monkey and said molecule comprises the following DNA sequence:

```
     1  ATGAACCGGGGAATCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTACTCCCA
   -25  MetAsnArgGlyIleProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro

                .         .         .         .         .         .
        GCAGTCACCCAGGGAAAGAAAGTGGTGCTGGGCAAGAAAGGGGATACAGTGGAACTGACC  120
        AlaValThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr   15

                .         .         .         .         .         .
   121  TGTACAGCTTCGCAGAAGAAGAACACACAATTCCACTGGAAAAACTCCAACCAGATAAAG
    16  CysThrAlaSerGlnLysLysAsnThrGlnPheHisTrpLysAsnSerAsnGlnIleLys

                .         .         .         .         .         .
        ATTCTGGGAATTCAGGGTCTCTTCTTAACTAAAGGTCCATCCAAGCTGAGCGATCGTGCT  240
        IleLeuGlyIleGlnGlyLeuPheLeuThrLysGlyProSerLysLeuSerAspArgAla   55

                .         .         .         .         .         .
   241  GACTCAAGAAAAAGCCTTTGGGACCAAGGATGCTTTTCCATGATCATCAAGAATCTTAAG
    56  AspSerArgLysSerLeuTrpAspGlnGlyCysPheSerMetIleIleLysAsnLeuLys

                .         .         .         .         .         .
        ATAGAAGACTCAGATACTTACATCTGTGAAGTGGAGAACAAGAAGGAGGAGGTGGAATTG  360
        IleGluAspSerAspThrTyrIleCysGluValGluAsnLysLysGluGluValGluLeu   95

                .         .         .         .         .         .
   361  CTGGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTGAGGGGCAAAGCCTGACC
    96  LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGluGlyGlnSerLeuThr
```

```
                .         .         .         .         .         .
        CTGACCTTGGAGAGCCCCCCTGGTAGTAGCCCCTCAGTGAAATGTAGGAGTCCAGGGGGT   480
        LeuThrLeuGluSerProProGlySerSerProSerValLysCysArgSerProGlyGly   135

                  .         .         .         .         .         .
481     AAAAACATACAGGGGGGGAGGACCATCTCTGTGCCTCAGCTGGAGCGCCAGGATAGTGGC
136     LysAsnIleGlnGlyGlyArgThrIleSerValProGlnLeuGluArgGlnAspSerGly

        .         .         .         .         .         .         .
        ACCTGGACATGCACCGTCTCGCAGGACCAGAAGACGGTGGAGTTCAAAATAGACATCGTG   600
        ThrTrpThrCysThrValSerGlnAspGlnLysThrValGluPheLysIleAspIleVal   175

                  .         .         .         .         .         .
601     GTGCTAGCTTTCCAGAAGGCCTCCAGCACAGTCTATAAGAAAGAGGGGGAACAGGTGGAG
176     ValLeuAlaPheGlnLysAlaSerSerThrValTyrLysLysGluGlyGluGlnValGlu

                  .         .         .         .         .         .
        TTCTCCTTCCCACTCGCCTTTACACTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGG   720
        PheSerPheProLeuAlaPheThrLeuGluLysLeuThrGlySerGlyGluLeuTrpTrp   215

                  .         .         .         .         .         .
721     CAGGCGGAGAGGGCCTCCTCCTCCAAGTCTTGGATTACCTTCGACCTGAAGAACAAGGAA
216     GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu

                  .         .         .         .         .         .
        GTGTCTGTAAAACGGGTTACCCAGGACCCCAAGCTCCAGATGGGCAAGAAGCTCCCGCTC   840
        ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu   255

                  .         .         .         .         .         .
841     CACCTCACCCTGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACGCTGGCC
256     HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla

                  .         .         .         .         .         .
        CTTGAAGCGAAAACAGGAAAGTTGCATCAGGAAGTGAACCTCGTGGTGATGAGAGCCACT   960
        LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr   295
```

```
                .         .         .         .         .         .
      961  CAGTTCCAGGAAAATTTGACCTGTGAAGTGTGGGGACCCACCTCCCCTAAGCTGACGCTG
      296  GlnPheGlnGluAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuThrLeu

                .         .         .         .         .         .
           AGCTTGAAACTGGAGAACAAGGGGGCAACGGTCTCGAAGCAGGCGAAGGCGGTGTGGGTG  1080
           SerLeuLysLeuGluAsnLysGlyAlaThrValSerLysGlnAlaLysAlaValTrpVal   335

                .         .         .         .         .         .
     1081  CTGAACCCTGAGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTA
      336  LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu

                .         .         .         .         .         .
           GAATCCAACATCAAGGTTGTGCCCACATGGCCCACCCCGGTGCAGCCAATGGCCCTGATT  1200
           GluSerAsnIleLysValValProThrTrpProThrProValGlnProMetAlaLeuIle   375

                .         .         .         .         .         .
     1201  GTGCTGGGGGGCGTTGCGGGCCTCCTGCTTTTCACTGGGCTAGGCATCTTCTTCTGTGTC
      376  ValLeuGlyGlyValAlaGlyLeuLeuLeuPheThrGlyLeuGlyIlePhePheCysVal

                .         .         .         .         .         .
           AGGTGCCGGCATCGAAGGCGTCAAGCAGAGCGGATGTCTCAGATCAAGAGACTCCTCAGT  1320
           ArgCysArgHisArgArgArgGlnAlaGluArgMetSerGlnIleLysArgLeuLeuSer   415

                .         .         .         .         .
     1321  GAAAAGAAGACCTGCCAGTGCCCTCACCGGTTTCAGAAGACATGTAGCCCCATT
      416  GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIle;
```

or a degenerate variant thereof, or wherein said non-human primate is the rhesus monkey and the said nucleic acid fragment comprises the following DNA sequence:

```
                .         .         .         .         .         .
        1  ATGAACCGGGGAATCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTACTCCCA
      -25  MetAsnArgGlyIleProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro

                .         .         .         .         .         .
           GCAGTCACCCAGGGAAAGAAAGTGGTGCTGGGCAAGAAAGGGGATACAGTGCAACTGACC   120
           AlaValThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr    15
```

```
                .       .       .       .       .       .
121  TGTACAGCTTCGCAGAAGAAGAACACACAATTCCACTGGAAAAACTCCAACCAGATAAAG
 16  CysThrAlaSerGlnLysLysAsnThrGlnPheHisTrpLysAsnSerAsnGlnIleLys

                .       .       .       .       .       .
     ATTCTGGGAATTCAGGGTCTCTTCTTAACTAAAGGTCCATCCAAGCTGAGCGATCGTGCT   240
     IleLeuGlyIleGlnGlyLeuPheLeuThrLysGlyProSerLysLeuSerAspArgAla    55

                        .       .       .       .       .
241  GACTCAAGAAAAAGCCTTTGGGACCAAGGATGCTTTTCCATGATCATCAAGAATCTTAAG
 56  AspSerArgLysSerLeuTrpAspGlnGlyCysPheSerMetIleIleLysAsnLeuLys

    .       .       .       .       .       .       .
     ATAGAAGACTCAGATACTTACATCTGTGAAGTGGAGAACAAGAAGGAGGAGGTGGAATTG   360
     IleGluAspSerAspThrTyrIleCysGluValGluAsnLysLysGluGluValGluLeu    95

                .       .       .       .
361  CTGGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTT
 96  LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu    ;
```

or a degenerate variant thereof, or wherein said non-human primate is the chimpanzee and said molecule comprises the following DNA sequence:

```
                .       .       .       .       .       .
  1  ATGAACCGGGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCACTCCTCCCA
-25  MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro

                .       .       .       .       .       .
     GCAGCCACTCAGGGAAAGAAAGTGGTGCTGGGCAAGAAAGGGGACACAGTGGAACTGACC   120
     AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr    15

                .       .       .       .       .       .
121  TGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGACAAAG
 16  CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnThrLys

                .       .       .       .       .       .
     ATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGTT   240
     IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgVal    55

                .       .       .       .       .       .
241  GACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTTACCCTGATCATCAAGAATCTTAAG
 56  AspSerArgArgSerLeuTrpAspGlnGlyAsnPheThrLeuIleIleLysAsnLeuLys
```

```
                                                                    .
                ATAGAAGACTCAGATACTTACATCTGTGAAGTGGGGGACCAGAAGGAGGAGGTGCAATTG   360
                IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu    95

                                                                    .
           361  CTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACC
            96  LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGlnGlyGlnSerLeuThr

                                                                    .
                CTGACCTTGGAGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGT   480
                LeuThrLeuGluSerProProGlySerSerProSerValGlnCysArgSerProArgGly   135

                                                                    .
           481  AAAAACATACAGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGC
           136  LysAsnIleGlnGlyGlyLysThrLeuSerValSerGlnLeuGluLeuGlnAspSerGly

                                                                    .
                ACCTGGACATGCACTGTCTTGCAGAACCAGAAGAAAGTGGAGTTCAAAATAGACATCGTG   600
                ThrTrpThrCysThrValLeuGlnAsnGlnLysLysValGluPheLysIleAspIleVal   175

                                                                    .
           601  GTGCTAGCTTTCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGGAG
           176  ValLeuAlaPheGlnLysAlaSerSerIleValTyrLysLysGluGlyGluGlnValGlu

                                                                    .
                TTCTCCTTCCCACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGG   720
                PheSerPheProLeuAlaPheThrValGluLysLeuThrGlySerGlyGluLeuTrpTrp   215

                                                                    .
           721  CAGGCGGAGAGGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAA
           216  GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu

                                                                    .
                GTGTCTGTAAAACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTC   840
                ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu   255

                                                                    .
           841  CACCTCACCCTGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACCCTGGCC
           256  HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla

                                                                    .
                CTTGAAGCGAAAACAGGAAAGTTGCATCAGGAAGTGAACCTCGTGGTGATGAGAGCCACT   960
                LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr   295
```

```
961  CAGCTCCAGAAAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTG
296  GlnLeuGlnLysAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuMetLeu

     AGCTTGAAACTGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGGCGGTGTGGGTG  1080
     SerLeuLysLeuGluAsnLysGluAlaLysValSerLysArgGluLysAlaValTrpVal   335

1081 CTGAACCCTGAGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTG
336  LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu

     GAATCCAACATCAAGGTTCTGCCCACATGGTCCACCCCGGTGCAGCCAATGGCCCTGATT  1200
     GluSerAsnIleLysValLeuProThrTrpSerThrProValGlnProMetAlaLeuIle   375

1201 GTGCTGGGGGGCGTCGCCGGCCTCCTGCTTTTCATTGGGCTAGGCATCTTCTTCTGTGTC
376  ValLeuGlyGlyValAlaGlyLeuLeuLeuPheIleGlyLeuGlyIlePhePheCysVal

     AGGTGCCGGCACCGAAGGCGCCAAGCACAGCGGATGTCTCAGATCAAGAGACTCCTCAGT  1320
     ArgCysArgHisArgArgArgGlnAlaGlnArgMetSerGlnIleLysArgLeuLeuSer   415

1321 GAGAAGAAGACCTGCCAGTGCCCTCACCGGTTTCAGAAGACATGTAGCCCCATT
416  GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIle  ;
```

or a degenerate variant thereof, or wherein said non-human primate is the chimpanzee and said nucleic acid fragment comprises the following DNA sequence:

```
1    ATGAACCGGGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCACTCCTCCCA
-25  MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro

     GCAGCCACTCAGGGAAAGAAAGTGGTGCTGGGCAAGAAAGGGGACACAGTGGAACTGACC  120
     AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr   15
```

```
                .         .         .         .         .         .
121  TGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGACAAAG
 16  CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnThrLys

                .         .         .         .         .         .
     ATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGTT   240
     IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgVal    55

                .         .         .         .         .         .
241  GACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTTACCCTGATCATCAAGAATCTTAAG
 56  AspSerArgArgSerLeuTrpAspGlnGlyAsnPheThrLeuIleIleLysAsnLeuLys

                .         .         .         .         .         .
     ATAGAAGACTCAGATACTTACATCTGTGAAGTGGGGGACCAGAAGGAGGAGGTGCAATTG   360
     IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu    95

                .         .         .         .
361  CTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTT
 96  LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu ;
```

or a degenerate variant thereof.

5. A recombinant DNA molecule comprising the following sequence:

```
                .         .         .         .         .         .
  1  ATGAACCGGGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCACTCCTCCCA

                .         .         .         .         .         .
     GCAGCCACTCAGGGAAAGAAAGTGGTGCTGGGCAAGAAAGGGGACACAGTGGAACTGACC   120

                .         .         .         .         .         .
121  TGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGAYAAAG

                .         .         .         .         .         .
     ATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGYT   240

                .         .         .         .         .         .
241  GACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTTMCCCTGATCATCAAGAATCTTAAG

                .         .         .         .         .         .
     ATAGAAGACTCAGATACTTACATCTGTGAAGTGGGGGACCAGAAGGAGGAGGTGCAATTG   360
```

```
                .         .         .         .         .         .
      361 CTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACC

                .         .         .         .         .         .
          CTGACCTTGGAGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGT   480

                .         .         .         .         .         .
      481 AAAAACATACAGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGC

                .         .         .         .         .         .
          ACCTGGACATGCACTGTCTTGCAGAACCAGAAGAAAGTGGAGTTCAAAATAGACATCGTG   600

                .         .         .         .         .         .
      601 GTGCTAGCTTTCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGGAG

                .         .         .         .         .         .
          TTCTCCTTCCCACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGG   720

                .         .         .         .         .         .
      721 CAGGCGGAGAGGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAA

                .         .         .         .         .         .
          GTGTCTGTAAAACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTC   840

                .         .         .         .         .         .
      841 CACCTCACCCTGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACCCTGGCC

                .         .         .         .         .         .
          CTTGAAGCGAAAACAGGAAAGTTGCATCAGGAAGTGAACCTCGTGGTGATGAGAGCCACT   960

                .         .         .         .         .         .
      961 CAGCTCCAGAAAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTG

                .         .         .         .         .         .
          AGCTTGAAACTGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGGCGGTGTGGGTG  1080

                .         .         .         .         .         .
     1081 CTGAACCCTGAGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTG

                .         .         .         .         .         .
          GAATCCAACATCAAGGTTCTGCCCACATGGTCCACCCCGGTGCAGCCAATGGCCCTGATT  1200
```

```
                .         .         .         .         .         .
1201  GTGCTGGGGGGCGTCGCCGGCCTCCTGCTTTTCATTGGGCTAGGCATCTTCTTCTGTGTC

                .         .         .         .         .         .
      AGGTGCCGGCACCGAAGGCGCCAAGCASAGCGGATGTCTCAGATCAAGAGACTCCTCAGT  1320

                .         .         .         .         .
1321  GAGAAGAAGACCTGCCAGTGCCCTCACCGGTTTCAGAAGACATGTAGCCCCATT;
```

wherein Y is C or T,
M is A or C, and
S is C or G;
or a degenerate variant thereof.

6. A nucleic acid molecule specifying glycosylated human CD4 with the cytoplasmic domain, comprising the following DNA sequence:

```
                .         .         .         .         .         .
   1  ATGAACCGGGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCA

                .         .         .         .         .         .
      GCAGCCACTCAGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACC   120

                .         .         .         .         .         .
 121  TGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGAYAAAG

                .         .         .         .         .         .
      ATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCT   240

                .         .         .         .         .         .
 241  GACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTTMCCCTGATCATCAAGAATCTTAAG

                .         .         .         .         .         .
      ATAGAAGACTCAGATACTTACATCTGTGAAGTGGGGGACCAGAAGGAGGAGGTGCAATTG   360

                .         .         .         .         .         .
 361  CTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACC

                .         .         .         .         .         .
      CTGACCTTGGAGAGCCCCCCTGGTAGTAGCCCCTCAGTGCAATGTAGGAGTCCAAGGGGT   480
```

```
 481  AAAAACATACAGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGC
      ACCTGGACATGCACTGTCTTGCAGAACCAGAAGAAGGTGGAGTTCAAAATAGACATCGTG   600
 601  GTGCTAGCTTTCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGGAG
      TTCTCCTTCCCACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGG   720
 721  CAGGCGGAGAGGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAA
      GTGTCTGTAAAACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTC   840
 841  CACCTCACCCTGCCCCAGGCCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACCCTGGCC
      CTTGAAGCGAAAACAGGAAAGTTGCATCAGGAAGTGAACCTGGTGGTGATGAGAGCCACT   960
 961  CAGCTCCAGAAAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTG
      AGCTTGAAACTGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGGCGGTGTGGGTG  1080
1081  CTGAACCCTGAGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCCTGCTG
      GAATCCAACATCAAGGTTCTGCCCACATGGTCCACCCCGGTGCAGCCAATGGCCCTGATT  1200
1201  GTGCTGGGGGGCGTCGCCGGCCTCCTGCTTTTCATTGGGCTAGGCATCTTCTTCTGTGTC
      AGGTGCCGGCACCGAAGGCGCCAAGCAGAGCGGATGTCTCAGATCAAGAGACTCCTCAGT  1320
1321  GAGAAGAAGACCTGCCAGTGCCCTCACCGGTTTCAGAAGACATGTAGCCCCATTTGA  1377
```

wherein Y is C or T, and

M is A or C;
or a degenerate variant thereof;
with the proviso that both Y is not T and M is not C at the same time.

7. A nucleic acid molecule specifying a glycosylated human CD4 fragment, comprising the following DNA sequence:

```
                .         .         .         .         .         .
      1 ATGAACCGGGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCA

                .         .         .         .         .         .
        GCAGCCACTCAGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAACTGACC    120

                .         .         .         .         .         .
    121 TGTACAGCTTCCCAGAAGAAGAGCATACAATTCCACTGGAAAAACTCCAACCAGAYAAAG

                .         .         .         .         .         .
        ATTCTGGGAAATCAGGGCTCCTTCTTAACTAAAGGTCCATCCAAGCTGAATGATCGCGCT    240

                .         .         .         .         .         .
    241 GACTCAAGAAGAAGCCTTTGGGACCAAGGAAACTTTMCCCTGATCATCAAGAATCTTAAG

                .         .         .         .         .         .
        ATAGAAGACTCAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTG    360

                .         .         .         .
    361 CTAGTGTTCGGATTGACTGCCAACTCTGACACCCACCTGCTT
```

wherein Y is C or T, and
M is A or C;
or a degenerate variant thereof;
with the proviso that both Y is not T and M is not C at the same time.

8. A nucleic acid molecule specifying a fusion protein, comprising:
1) the nucleic acid molecule of claim 1, linked to
2) a nucleic acid molecule specifying an immunoglobulin heavy chain, preferably of the class IgM, IgG1 or IgG3,
wherein the nucleic acid sequence which specifies the variable region of said immunoglobulin heavy chain has been replaced with said nucleic acid molecule specifying said fragment.

9. A nucleic acid molecule specifying a fusion protein, comprising:
1) a nucleic acid molecule specifying a non-human primate CD4, or HIV or SIV gp120 binding fragment thereof, linked to
2) a nucleic acid molecule specifying an immunoglobulin light chain, preferably of the class IgM, IgG1 or IgG3,
wherein the nucleic acid sequence which specifies the variable region of said immunoglobulin light chain has been replaced with said nucleic acid molecule specifying said fragment.

10. A nucleic acid molecule specifying a fusion protein, comprising:
1) a nucleic acid molecule specifying a non-human primate CD4, or HIV or SIV gp120 binding fragment thereof, linked to
2) a nucleic acid molecule specifying a cytotoxic polypeptide.

11. A vector comprising the nucleic acid molecule of any one of claims 1 or 4 to 10.

12. A host transformed with the vector of claim 11, especially a host transformed with a vector comprising the nucleic acid molecule of claim 8, wherein said host expresses an immunoglobulin light chain together with the expression product of nucleic acid molecule to give an immunoglobulin-like molecule which binds

to HIV or SIV gp120, or a host transformed with a vector comprising the nucleic acid molecule of claim 9, wherein said host expresses an immunoglobulin heavy chain together with the expression product of nucleic acid molecule to give an immunoglobulin-like molecule which binds to HIV or SIV gp120, and wherein said immunoglobulin heavy chain is preferably of the immunoglobulin class IgM, IgG1 or IgG3.

13. A method of producing non-human primate CD4, or fragment thereof which binds to HIV or SIV gp120, which comprises

cultivating in a nutrient medium under protein-producing conditions, a host strain transformed with a vector comprising the nucleic acid molecule of claim 1, said vector further comprising expression signals which are recognized by said host strain and direct expression of said non-human primate CD4, and recovering the non-human primate CD4 so produced.

14. A method of producing a fusion protein comprising non-human primate CD4, or fragment thereof which binds to gp120, and an immunoglobulin heavy chain, wherein the variable region of the immunoglobulin chain has been substituted with non-human primate CD4, or fragment thereof which binds to HIV or SIV gp120, which comprises

cultivating in a nutrient medium under protein-producing conditions, a host strain transformed with a vector comprising the nucleic acid molecule of claim 8, said vector further comprising expression signals which are recognized by said host strain and direct expression of said fusion protein, and

recovering the fusion protein so produced, and wherein said host strain preferably is a myeloma cell line which produces immunoglobulin light chains and said fusion protein comprises an immunoglobulin heavy chain of the class IgM, IgG1 or IgG3, wherein an immunoglobulin-like molecule comprising said fusion protein is produced.

15. A method of producing a fusion protein comprising non-human primate CD4, or fragment thereof which binds to HIV or SIV gp120, and an immunoglobulin light chain, wherein the variable region of theimmunoglobulin chain has been substituted with non-human primate CD4, or fragment thereof which binds to HIV or SIV gp120, which comprises:

cultivating in a nutrient medium under protein-producing conditions, a host strain transformed with a vector comprising the nucleic acid molecule of claim 9, said vector further comprising expression signals which are recognized by said host strain and direct expression of said fusion protein, and

recovering the fusion protein so produced, and wherein said host preferably produces immunoglobulin heavy chains of the class IgM, IgG1 and IgG3 together with said fusion protein to give an immunoglobulin-like molecule which binds to HIV or SIV gp120.

16. Substantially pure non-human primate CD4, especially a substantially pure non-human primate CD4, wherein said non-human primate is the rhesus monkey, comprising the following amino acid sequence:

MetAsnArgGlyIleProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaValThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysAsnThrGlnPheHisTrpLysAsnSerAsnGlnIleLys
IleLeuGlyIleGlnGlyLeuPheLeuThrLysGlyProSerLysLeuSerAspArgAla
AspSerArgLysSerLeuTrpAspGlnGlyCysPheSerMetIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGluAsnLysLysGluGluValGluLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGluGlyGlnSerLeuThr
LeuThrLeuGluSerProProGlySerSerProSerValLysCysArgSerProGlyGly
LysAsnIleGlnGlyGlyArgThrIleSerValProGlnLeuGluArgGlnAspSerGly
ThrTrpThrCysThrValSerGlnAspGlnLysThrValGluPheLysIleAspIleVal
ValLeuAlaPheGlnLysAlaSerSerThrValTyrLysLysGluGlyGluGlnValGlu
PheSerPheProLeuAlaPheThrLeuGluLysLeuThrGlySerGlyGluLeuTrpTrp
GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu
ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu
HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla
LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr

GlnPheGlnGluAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuThrLeu
SerLeuLysLeuGluAsnLysGlyAlaThrValSerLysGlnAlaLysAlaValTrpVal
LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu
GluSerAsnIleLysValValProThrTrpProThrProValGlnProMetAlaLeuIle
ValLeuGlyGlyValAlaGlyLeuLeuLeuPheThrGlyLeuGlyIlePhePheCysVal
ArgCysArgHisArgArgArgGlnAlaGluArgMetSerGlnIleLysArgLeuLeuSer
GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIle,

or a substantially pure non-human CD4, wherein said non-human primate is the chimpanzee, comprising the following amino acid sequence.

MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnThrLys
IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgVal
AspSerArgArgSerLeuTrpAspGlnGlyAsnPheThrLeuIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGlnGlyGlnSerLeuThr
LeuThrLeuGluSerProProGlySerSerProSerValGlnCysArgSerProArgGly
LysAsnIleGlnGlyGlyLysThrLeuSerValSerGlnLeuGluLeuGlnAspSerGly
ThrTrpThrCysThrValLeuGlnAsnGlnLysLysValGluPheLysIleAspIleVal
ValLeuAlaPheGlnLysAlaSerSerIleValTyrLysLysGluGlyGluGlnValGlu
PheSerPheProLeuAlaPheThrValGluLysLeuThrGlySerGlyGluLeuTrpTrp
GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu
ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu
HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla
LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr
GlnLeuGlnLysAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuMetLeu
SerLeuLysLeuGluAsnLysGluAlaLysValSerLysArgGluLysAlaValTrpVal
LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu
GluSerAsnIleLysValLeuProThrTrpSerThrProValGlnProMetAlaLeuIle
ValLeuGlyGlyValAlaGlyLeuLeuLeuPheIleGlyLeuGlyIlePhePheCysVal
ArgCysArgHisArgArgArgGlnAlaGlnArgMetSerGlnIleLysArgLeuLeuSer
GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIle;

or the glycosylated derivative thereof, or a substantially pure non-human CD4 comprising the following amino acid sequence:

MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGln-@-Lys
IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArg-#-
AspSerArgArgSerLeuTrpAspGlnGlyAsnPhe-$-LeuIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGlnGlyGlnSerLeuThr
LeuThrLeuGluSerProProGlySerSerProSerValGlnCysArgSerProArgGly
LysAsnIleGlnGlyGlyLysThrLeuSerValSerGlnLeuGluLeuGlnAspSerGly
ThrTrpThrCysThrValLeuGlnAsnGlnLysLysValGluPheLysIleAspIleVal
ValLeuAlaPheGlnLysAlaSerSerIleValTyrLysLysGluGlyGluGlnValGlu
PheSerPheProLeuAlaPheThrValGluLysLeuThrGlySerGlyGluLeuTrpTrp
GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu
ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu
HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla
LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr
GlnLeuGlnLysAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuMetLeu
SerLeuLysLeuGluAsnLysGluAlaLysValSerLysArgGluLysAlaValTrpVal
LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu
GluSerAsnIleLysValLeuProThrTrpSerThrProValGlnProMetAlaLeuIle
ValLeuGlyGlyValAlaGlyLeuLeuLeuPheIleGlyLeuGlyIlePhePheCysVal
ArgCysArgHisArgArgArgGlnAla-%-ArgMetSerGlnIleLysArgLeuLeuSer
GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIle,

wherein
-@- is Thr or Ile,
-#- is Val or Ala,
-$- is Thr or Pro, and
-%- is Gln or Glu;

or the glycosylated derivative thereof, or a substantially pure non-human CD4 comprising the following amino acid sequence:

MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGln-@-Lys
IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArg-#-
AspSerArgArgSerLeuTrpAspGlnGlyAsnPhe-$-LeuIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu

wherein
-@- is Thr or Ile,
-#- is Val or Ala, and
-$- is Thr or Pro; or
the glycosylated derivative thereof.

17. A gp120 binding molecule comprising the following amino acid sequence:

MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGln-@-Lys
IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgAla
AspSerArgArgSerLeuTrpAspGlnGlyAsnPhe-$-LeuIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGluAspGlnLysGluGluValGlnLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeuGlnGlyGlnSerLeuThr
LeuThrLeuGluSerProProGlySerSerProSerValGlnCysArgSerProArgGly
LysAsnIleGlnGlyGlyLysThrLeuSerValSerGlnLeuGluLeuGlnAspSerGly
ThrTrpThrCysThrValLeuGlnAsnGlnLysLysValGluPheLysIleAspIleVal
ValLeuAlaPheGlnLysAlaSerSerIleValTyrLysLysGluGlyGluGlnValGlu
PheSerPheProLeuAlaPheThrValGluLysLeuThrGlySerGlyGluLeuTrpTrp
GlnAlaGluArgAlaSerSerSerLysSerTrpIleThrPheAspLeuLysAsnLysGlu
ValSerValLysArgValThrGlnAspProLysLeuGlnMetGlyLysLysLeuProLeu
HisLeuThrLeuProGlnAlaLeuProGlnTyrAlaGlySerGlyAsnLeuThrLeuAla
LeuGluAlaLysThrGlyLysLeuHisGlnGluValAsnLeuValValMetArgAlaThr

GlnLeuGlnLysAsnLeuThrCysGluValTrpGlyProThrSerProLysLeuMetLeu
SerLeuLysLeuGluAsnLysGluAlaLysValSerLysArgGluLysAlaValTrpVal
LeuAsnProGluAlaGlyMetTrpGlnCysLeuLeuSerAspSerGlyGlnValLeuLeu
GluSerAsnIleLysValLeuProThrTrpSerThrProValGlnProMetAlaLeuIle
ValLeuGlyGlyValAlaGlyLeuLeuLeuPheIleGlyLeuGlyIlePhePheCysVal
ArgCysArgHisArgArgArgGlnAlaGluArgMetSerGlnIleLysArgLeuLeuSer
GluLysLysThrCysGlnCysProHisArgPheGlnLysThrCysSerProIle,

wherein
-@- is Thr or Ile, and
-$- is Thr or Pro; or
the glycosylated derivative thereof;
with the proviso that at least one of -@- and -$- is Thr,
or comprising the following amino acid sequence:

MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGln-@-Lys
IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgAla
AspSerArgArgSerLeuTrpAspGlnGlyAsnPhe-$-LeuIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGluAspGlnLysGluGluValGlnLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu

wherein
-@- is Thr or Ile, and
-$- is Thr or Pro; or
the glycosylated derivative thereof;
with the proviso that at least one of -@- and -$- is Thr,
and wherein the gp120 binding molecule is preferably linked to a cytotoxic polypeptide, radiolabeled or NMR imaging agent.

18. A non-human primate, preferably rhesus monkey or the chimpanzee, CD4 fragment which is capable of binding to HIV or SIV gp120, which preferably is soluble in aqueous solution.

19. The non-human primate CD4 fragment of claim 18, wherein said non-human primate is the rhesus monkey, comprising the following amino acid sequence:

MetAsnArgGlyIleProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaValThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysAsnThrGlnPheHisTrpLysAsnSerAsnGlnIleLys
IleLeuGlyIleGlnGlyLeuPheLeuThrLysGlyProSerLysLeuSerAspArgAla
AspSerArgLysSerLeuTrpAspGlnGlyCysPheSerMetIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGluAsnLysLysGluGluValGluLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu,

or wherein said non-human primate is the chimpanzee, comprising the following amino acid sequence:

MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnThrLys
IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgVal
AspSerArgArgSerLeuTrpAspGlnGlyAsnPheThrLeuIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu;

or the glycosylated derivative thereof.

20. A fusion protein comprising non-human primate CD4, or fragment thereof which is capable of binding to HIV or SIV gp120, fused at the C-terminus to a second protein which comprises an immunoglobulin heavy chain of the class IgM, IgG1 or IgG3, wherein the variable region of said heavy chain immunoglobulin has been replaced with CD4, or HIV gp120-binding fragment thereof, which fusion protein preferably is detectably labeled, or linked to a cytotoxic polypeptide, preferably comprising ricin or diphtheria toxin, radiolabel or NMR imaging agent.

21. A fusion protein comprising non-human primate CD4, or fragment thereof which binds to HIV or SIV gp120, fused at the terminus to a second protein comprising an immunoglobulin light chain wherein the variable region has been deleted, which preferably is detectably labeled or linked to a cytotoxic polypeptide, especially comprising ricin or diphtheria toxin, radiolabel or NMR imaging agent.

22. The fusion protein of claim 19 or 20, wherein said CD4 fragment is derived from the rhesus monkey, comprising the following amino acid sequence:

MetAsnArgGlyIleProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaValThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysAsnThrGlnPheHisTrpLysAsnSerAsnGlnIleLys
IleLeuGlyIleGlnGlyLeuPheLeuThrLysGlyProSerLysLeuSerAspArgAla
AspSerArgLysSerLeuTrpAspGlnGlyCysPheSerMetIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGluAsnLysLysGluGluValGluLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu, or

wherein said CD4 fragment is derived from the chimpanzee, comprising the following amino acid sequence:

MetAsnArgGlyValProPheArgHisLeuLeuLeuValLeuGlnLeuAlaLeuLeuPro
AlaAlaThrGlnGlyLysLysValValLeuGlyLysLysGlyAspThrValGluLeuThr
CysThrAlaSerGlnLysLysSerIleGlnPheHisTrpLysAsnSerAsnGlnThrLys
IleLeuGlyAsnGlnGlySerPheLeuThrLysGlyProSerLysLeuAsnAspArgVal
AspSerArgArgSerLeuTrpAspGlnGlyAsnPheThrLeuIleIleLysAsnLeuLys
IleGluAspSerAspThrTyrIleCysGluValGlyAspGlnLysGluGluValGlnLeu
LeuValPheGlyLeuThrAlaAsnSerAspThrHisLeuLeu.

23. An immunoglobulin-like molecule, comprising the fusion protein of claim 19 and an immunoglobulin light chain, which immunoglobulin-like molecule preferably is detectably labelled or linked to a cytotoxic polypeptide, radiolabel or NMR imaging agent.

24. An immunoglobulin-like molecule comprising the fusion protein of claim 21 and an immunoglobulin

heavy chain of the class IgM, IgG1 or IgG3, which immunoglobulin-like molecule preferably is detectably labeled or wherein said fusion protein is linked to a cytotoxic polypeptide, radiolabel or NMR imaging agent.

25. A non-human primate CD4 molecule, or an HIV or SIV gp120 binding fragment thereof, linked to a cytotoxic polypeptide, radiolabel or NMR imaging agent.

26. A complex, comprising

a) HIV or SIV gp120 and

b) substantially pure non-human primate CD4, or an HIV or SIV gp120 binding non-human primate CD4 fragment, or an HIV or SIV gp120 binding non-human primate CD4 soluble fragment, or the fusion protein of claim 20 or 21, or the gp120 binding molecule of claim 17.

27. The complex of claim 26, wherein said gp120 is a part of an HIV or SIV, is expressed on the surface of an HIV or SIV-infected cell or is present in solution.

28. A method for the detection of HIV or SIV gp120 in a sample, comprising

(a) contacting a sample suspected of containing HIV or SIV gp120 with the fusion protein of claim 20 or 21, and

(b) detecting whether a complex is formed.

29. A method for the detection of HIV or SIV gp120 in a sample, comprising

(a) contacting a sample suspected of containing HIV or SIV gp120 with non-human primate CD4, or fragment thereof which is capable of binding to HIV or SIV gp120, and wherein preferably said non-human primate CD4 or fragment thereof is detectably labeled,

(b) detecting whether a complex has formed.

30. A method for the detection of HIV or SIV gp120 in a sample, comprising

(a) contacting a sample suspected of containing HIV or SIV gp120 with the gp120 binding molecule of claim 17, which preferably is detectably labeled; and

(b) detecting whether a complex has formed.

31. A pharmaceutical composition comprising a therapeutically effective amount of substantially pure non-human primate CD4 or a therapeutically effective amount of a non-human primate CD4 fragment which is capable of binding to HIV or SIV gp120, and preferably soluble in aqueous solution, or a therapeutically effective amount of the gp120 binding molecule of claim 17; and a pharmaceutically acceptable carrier.

SEQ ID NO.: 1
SEQUENCE TYPE: Nucleotide with corresponding protein
SEQUENCE LENGTH: 1374 bases

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: None

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ATG AAC CGG GGA ATC CCT TTT AGG CAC TTG CTT CTG GTG CTG CAA CTG      48
Met Asn Arg Gly Ile Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
                  5                  10                  15

GCG CTA CTC CCA GCA GTC ACC CAG GGA AAG AAA GTG GTG CTG GGC AAG      96
Ala Leu Leu Pro Ala Val Thr Gln Gly Lys Lys Val Val Leu Gly Lys
             20                  25                  30

AAA GGG GAT ACA GTG GAA CTG ACC TGT ACA GCT TCG CAG AAG AAG AAC     144
Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Asn
         35                  40                  45

ACA CAA TTC CAC TGG AAA AAC TCC AAC CAG ATA AAG ATT CTG GGA ATT     192
Thr Gln Phe His Trp Lys Asn Ser Asn Gln Ile Lys Ile Leu Gly Ile
     50                  55                  60

CAG GGT CTC TTC TTA ACT AAA GGT CCA TCC AAG CTG AGC GAT CGT GCT     240
Gln Gly Leu Phe Leu Thr Lys Gly Pro Ser Lys Leu Ser Asp Arg Ala
 65                  70                  75                  80

GAC TCA AGA AAA AGC CTT TGG GAC CAA GGA TGC TTT TCC ATG ATC ATC     288
Asp Ser Arg Lys Ser Leu Trp Asp Gln Gly Cys Phe Ser Met Ile Ile
                 85                  90                  95

AAG AAT CTT AAG ATA GAA GAC TCA GAT ACT TAC ATC TGT GAA GTG GAG     336
Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Glu
            100                 105                 110

AAC AAG AAG GAG GAG GTG GAA TTG CTG GTG TTC GGA TTG ACT GCC AAC     384
Asn Lys Lys Glu Glu Val Glu Leu Leu Val Phe Gly Leu Thr Ala Asn
        115                 120                 125

TCT GAC ACC CAC CTG CTT GAG GGG CAA AGC CTG ACC CTG ACC TTG GAG     432
Ser Asp Thr His Leu Leu Glu Gly Gln Ser Leu Thr Leu Thr Leu Glu
    130                 135                 140

AGC CCC CCT GGT AGT AGC CCC TCA GTG AAA TGT AGG AGT CCA GGG GGT     480
Ser Pro Pro Gly Ser Ser Pro Ser Val Lys Cys Arg Ser Pro Gly Gly
145                 150                 155                 160

AAA AAC ATA CAG GGG GGG AGG ACC ATC TCT GTG CCT CAG CTG GAG CGC     528
Lys Asn Ile Gln Gly Gly Arg Thr Ile Ser Val Pro Gln Leu Glu Arg
                165                 170                 175
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```
CAG GAT AGT GGC ACC TGG ACA TGC ACC GTC TCG CAG GAC CAG AAG ACG     576
Gln Asp Ser Gly Thr Trp Thr Cys Thr Val Ser Gln Asp Gln Lys Thr
            180                 185                 190

GTG GAG TTC AAA ATA GAC ATC GTG GTG CTA GCT TTC CAG AAG GCC TCC     624
Val Glu Phe Lys Ile Asp Ile Val Val Leu Ala Phe Gln Lys Ala Ser
        195                 200                 205

AGC ACA GTC TAT AAG AAA GAG GGG GAA CAG GTG GAG TTC TCC TTC CCA     672
Ser Thr Val Tyr Lys Lys Glu Gly Glu Gln Val Glu Phe Ser Phe Pro
    210                 215                 220

CTC GCC TTT ACA CTT GAA AAG CTG ACG GGC AGT GGC GAG CTG TGG TGG     720
Leu Ala Phe Thr Leu Glu Lys Leu Thr Gly Ser Gly Glu Leu Trp Trp
225                 230                 235                 240

CAG GCG GAG AGG GCC TCC TCC TCC AAG TCT TGG ATT ACC TTC GAC CTG     768
Gln Ala Glu Arg Ala Ser Ser Ser Lys Ser Trp Ile Thr Phe Asp Leu
                245                 250                 255

AAG AAC AAG GAA GTG TCT GTA AAA CGG GTT ACC CAG GAC CCC AAG CTC     816
Lys Asn Lys Glu Val Ser Val Lys Arg Val Thr Gln Asp Pro Lys Leu
            260                 265                 270

CAG ATG GGC AAG AAG CTC CCG CTC CAC CTC ACC CTG CCC CAG GCC TTG     864
Gln Met Gly Lys Lys Leu Pro Leu His Leu Thr Leu Pro Gln Ala Leu
        275                 280                 285

CCT CAG TAT GCT GGC TCT GGA AAC CTC ACG CTG GCC CTT GAA GCG AAA     912
Pro Gln Tyr Ala Gly Ser Gly Asn Leu Thr Leu Ala Leu Glu Ala Lys
    290                 295                 300

ACA GGA AAG TTG CAT CAG GAA GTG AAC CTC GTG GTG ATG AGA GCC ACT     960
Thr Gly Lys Leu His Gln Glu Val Asn Leu Val Val Met Arg Ala Thr
305                 310                 315                 320

CAG TTC CAG GAA AAT TTG ACC TGT GAA GTG TGG GGA CCC ACC TCC CCT    1008
Gln Phe Gln Glu Asn Leu Thr Cys Glu Val Trp Gly Pro Thr Ser Pro
                325                 330                 335

AAG CTG ACG CTG AGC TTG AAA CTG GAG AAC AAG GGG GCA ACG GTC TCG    1056
Lys Leu Thr Leu Ser Leu Lys Leu Glu Asn Lys Gly Ala Thr Val Ser
            340                 345                 350

AAG CAG GCG AAG GCG GTG TGG GTG CTG AAC CCT GAG GCG GGG ATG TGG    1104
Lys Gln Ala Lys Ala Val Trp Val Leu Asn Pro Glu Ala Gly Met Trp
        355                 360                 365

CAG TGT CTG CTG AGT GAC TCG GGA CAG GTC CTG CTA GAA TCC AAC ATC    1152
Gln Cys Leu Leu Ser Asp Ser Gly Gln Val Leu Leu Glu Ser Asn Ile
    370                 375                 380
```

```
AAG GTT GTG CCC ACA TGG CCC ACC CCG GTG CAG CCA ATG GCC CTG ATT   1200
Lys Val Val Pro Thr Trp Pro Thr Pro Val Gln Pro Met Ala Leu Ile
385                 390                 395                 400

GTG CTG GGG GGC GTT GCG GGC CTC CTG CTT TTC ACT GGG CTA GGC ATC   1248
Val Leu Gly Gly Val Ala Gly Leu Leu Leu Phe Thr Gly Leu Gly Ile
                405                 410                 415

TTC TTC TGT GTC AGG TGC CGG CAT CGA AGG CGT CAA GCA GAG CGG ATG   1296
Phe Phe Cys Val Arg Cys Arg His Arg Arg Arg Gln Ala Glu Arg Met
            420                 425                 430

TCT CAG ATC AAG AGA CTC CTC AGT GAA AAG AAG ACC TGC CAG TGC CCT   1344
Ser Gln Ile Lys Arg Leu Leu Ser Glu Lys Lys Thr Cys Gln Cys Pro
        435                 440                 445

CAC CGG TTT CAG AAG ACA TGT AGC CCC ATT                          1374
His Arg Phe Gln Lys Thr Cys Ser Pro Ile
    450                 455
```

SEQ ID NO.: 2
SEQUENCE TYPE: Nucleotide with corresponding protein
SEQUENCE LENGTH: 401 bases

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: None

```
ATG AAC CGG GGA ATC CCT TTT AGG CAC TTG CTT CTG GTG CTG CAA CTG      48
Met Asn Arg Gly Ile Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
                  5                  10                  15

GCG CTA CTC CCA GCA GTC ACC CAG GGA AAG AAA GTG GTG CTG GGC AAG      96
Ala Leu Leu Pro Ala Val Thr Gln Gly Lys Lys Val Val Leu Gly Lys
             20                  25                  30

AAA GGG GAT ACA GTG GAA CTG ACC TGT ACA GCT TCG CAG AAG AAG AAC     144
Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Asn
         35                  40                  45

ACA CAA TTC CAC TGG AAA AAC TCC AAC CAG ATA AAG ATT CTG GGA ATT     192
Thr Gln Phe His Trp Lys Asn Ser Asn Gln Ile Lys Ile Leu Gly Ile
     50                  55                  60

CAG GGT CTC TTC TTA ACT AAA GGT CCA TCC AAG CTG AGC GAT CGT GCT     240
Gln Gly Leu Phe Leu Thr Lys Gly Pro Ser Lys Leu Ser Asp Arg Ala
65                  70                  75                  80

GAC TCA AGA AAA AGC CTT TGG GAC CAA GGA TGC TTT TCC ATG ATC ATC     288
Asp Ser Arg Lys Ser Leu Trp Asp Gln Gly Cys Phe Ser Met Ile Ile
                 85                  90                  95

AAG AAT CTT AAG ATA GAA GAC TCA GAT ACT TAC ATC TGT GAA GTG GAG     336
Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Glu
            100                 105                 110

AAC AAG AAG GAG GAG GTG GAA TTG CTG GTG TTC GGA TTG ACT GCC AAC     384
Asn Lys Lys Glu Glu Val Glu Leu Leu Val Phe Gly Leu Thr Ala Asn
        115                 120                 125

TCT GAC ACC CAC CTG CTT                                             402
Ser Asp Thr His Leu Leu
    130
```

SEQ ID NO.: 3
SEQUENCE TYPE: Nucleic acid with corresponding protein
SEQUENCE LENGTH: 1374 bases

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: None

```
ATG AAC CGG GGA GTC CCT TTT AGG CAC TTG CTT CTG GTG CTG CAA CTG      48
Met Asn Arg Gly Val Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
                5                  10                  15

GCA CTC CTC CCA GCA GCC ACT CAG GGA AAG AAA GTG GTG CTG GGC AAG      96
Ala Leu Leu Pro Ala Ala Thr Gln Gly Lys Lys Val Val Leu Gly Lys
            20                  25                  30

AAA GGG GAC ACA GTG GAA CTG ACC TGT ACA GCT TCC CAG AAG AAG AGC     144
Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Ser
        35                  40                  45

ATA CAA TTC CAC TGG AAA AAC TCC AAC CAG ACA AAG ATT CTG GGA AAT     192
Ile Gln Phe His Trp Lys Asn Ser Asn Gln Thr Lys Ile Leu Gly Asn
    50                  55                  60

CAG GGC TCC TTC TTA ACT AAA GGT CCA TCC AAG CTG AAT GAT CGC GTT     240
Gln Gly Ser Phe Leu Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Val
65                  70                  75                  80

GAC TCA AGA AGA AGC CTT TGG GAC CAA GGA AAC TTT ACC CTG ATC ATC     288
Asp Ser Arg Arg Ser Leu Trp Asp Gln Gly Asn Phe Thr Leu Ile Ile
                85                  90                  95

AAG AAT CTT AAG ATA GAA GAC TCA GAT ACT TAC ATC TGT GAA GTG GGG     336
Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Gly
            100                 105                 110

GAC CAG AAG GAG GAG GTG CAA TTG CTA GTG TTC GGA TTG ACT GCC AAC     384
Asp Gln Lys Glu Glu Val Gln Leu Leu Val Phe Gly Leu Thr Ala Asn
        115                 120                 125

TCT GAC ACC CAC CTG CTT CAG GGG CAG AGC CTG ACC CTG ACC TTG GAG     432
Ser Asp Thr His Leu Leu Gln Gly Gln Ser Leu Thr Leu Thr Leu Glu
    130                 135                 140

AGC CCC CCT GGT AGT AGC CCC TCA GTG CAA TGT AGG AGT CCA AGG GGT     480
Ser Pro Pro Gly Ser Ser Pro Ser Val Gln Cys Arg Ser Pro Arg Gly
145                 150                 155                 160

AAA AAC ATA CAG GGG GGG AAG ACC CTC TCC GTG TCT CAG CTG GAG CTC     528
Lys Asn Ile Gln Gly Gly Lys Thr Leu Ser Val Ser Gln Leu Glu Leu
                165                 170                 175
```

```
CAG GAT AGT GGC ACC TGG ACA TGC ACT GTC TTG CAG AAC CAG AAG AAA     576
Gln Asp Ser Gly Thr Trp Thr Cys Thr Val Leu Gln Asn Gln Lys Lys
            180                 185                 190

GTG GAG TTC AAA ATA GAC ATC GTG GTG CTA GCT TTC CAG AAG GCC TCC     624
Val Glu Phe Lys Ile Asp Ile Val Val Leu Ala Phe Gln Lys Ala Ser
        195                 200                 205

AGC ATA GTC TAT AAG AAA GAG GGG GAA CAG GTG GAG TTC TCC TTC CCA     672
Ser Ile Val Tyr Lys Lys Glu Gly Glu Gln Val Glu Phe Ser Phe Pro
    210                 215                 220

CTC GCC TTT ACA GTT GAA AAG CTG ACG GGC AGT GGC GAG CTG TGG TGG     720
Leu Ala Phe Thr Val Glu Lys Leu Thr Gly Ser Gly Glu Leu Trp Trp
225                 230                 235                 240

CAG GCG GAG AGG GCT TCC TCC TCC AAG TCT TGG ATC ACC TTT GAC CTG     768
Gln Ala Glu Arg Ala Ser Ser Ser Lys Ser Trp Ile Thr Phe Asp Leu
                245                 250                 255

AAG AAC AAG GAA GTG TCT GTA AAA CGG GTT ACC CAG GAC CCT AAG CTC     816
Lys Asn Lys Glu Val Ser Val Lys Arg Val Thr Gln Asp Pro Lys Leu
            260                 265                 270

CAG ATG GGC AAG AAG CTC CCG CTC CAC CTC ACC CTG CCC CAG GCC TTG     864
Gln Met Gly Lys Lys Leu Pro Leu His Leu Thr Leu Pro Gln Ala Leu
        275                 280                 285

CCT CAG TAT GCT GGC TCT GGA AAC CTC ACC CTG GCC CTT GAA GCG AAA     912
Pro Gln Tyr Ala Gly Ser Gly Asn Leu Thr Leu Ala Leu Glu Ala Lys
    290                 295                 300

ACA GGA AAG TTG CAT CAG GAA GTG AAC CTC GTG GTG ATG AGA GCC ACT     960
Thr Gly Lys Leu His Gln Glu Val Asn Leu Val Val Met Arg Ala Thr
305                 310                 315                 320

CAG CTC CAG AAA AAT TTG ACC TGT GAG GTG TGG GGA CCC ACC TCC CCT    1008
Gln Leu Gln Lys Asn Leu Thr Cys Glu Val Trp Gly Pro Thr Ser Pro
                325                 330                 335

AAG CTG ATG CTG AGC TTG AAA CTG GAG AAC AAG GAG GCA AAG GTC TCG    1056
Lys Leu Met Leu Ser Leu Lys Leu Glu Asn Lys Glu Ala Lys Val Ser
            340                 345                 350

AAG CGG GAG AAG GCG GTG TGG GTG CTG AAC CCT GAG GCG GGG ATG TGG    1104
Lys Arg Glu Lys Ala Val Trp Val Leu Asn Pro Glu Ala Gly Met Trp
        355                 360                 365

CAG TGT CTG CTG AGT GAC TCG GGA CAG GTC CTG CTG GAA TCC AAC ATC    1152
Gln Cys Leu Leu Ser Asp Ser Gly Gln Val Leu Leu Glu Ser Asn Ile
    370                 375                 380
```

```
AAG GTT CTG CCC ACA TGG TCC ACC CCG GTG CAG CCA ATG GCC CTG ATT   1200
Lys Val Leu Pro Thr Trp Ser Thr Pro Val Gln Pro Met Ala Leu Ile
385                 390                 395                 400

GTG CTG GGG GGC GTC GCC GGC CTC CTG CTT TTC ATT GGG CTA GGC ATC   1248
Val Leu Gly Gly Val Ala Gly Leu Leu Leu Phe Ile Gly Leu Gly Ile
                405                 410                 415

TTC TTC TGT GTC AGG TGC CGG CAC CGA AGG CGC CAA GCA CAG CGG ATG   1296
Phe Phe Cys Val Arg Cys Arg His Arg Arg Arg Gln Ala Gln Arg Met
            420                 425                 430

TCT CAG ATC AAG AGA CTC CTC AGT GAG AAG AAG ACC TGC CAG TGC CCT   1344
Ser Gln Ile Lys Arg Leu Leu Ser Glu Lys Lys Thr Cys Gln Cys Pro
        435                 440                 445

CAC CGG TTT CAG AAG ACA TGT AGC CCC ATT                          1374
His Arg Phe Gln Lys Thr Cys Ser Pro Ile
    450                 455
```

SEQ ID NO.: 4
SEQUENCE TYPE: Nucleic acid with corresponding protein
SEQUENCE LENGTH: 402 bases

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: None

```
ATG AAC CGG GGA GTC CCT TTT AGG CAC TTG CTT CTG GTG CTG CAA CTG      48
Met Asn Arg Gly Val Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
                  5                  10                  15

GCA CTC CTC CCA GCA GCC ACT CAG GGA AAG AAA GTG GTG CTG GGC AAG      96
Ala Leu Leu Pro Ala Ala Thr Gln Gly Lys Lys Val Val Leu Gly Lys
             20                  25                  30

AAA GGG GAC ACA GTG GAA CTG ACC TGT ACA GCT TCC CAG AAG AAG AGC     144
Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Ser
         35                  40                  45

ATA CAA TTC CAC TGG AAA AAC TCC AAC CAG ACA AAG ATT CTG GGA AAT     192
Ile Gln Phe His Trp Lys Asn Ser Asn Gln Thr Lys Ile Leu Gly Asn
     50                  55                  60

CAG GGC TCC TTC TTA ACT AAA GGT CCA TCC AAG CTG AAT GAT CGC GTT     240
Gln Gly Ser Phe Leu Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Val
 65                  70                  75                  80

GAC TCA AGA AGA AGC CTT TGG GAC CAA GGA AAC TTT ACC CTG ATC ATC     288
Asp Ser Arg Arg Ser Leu Trp Asp Gln Gly Asn Phe Thr Leu Ile Ile
                 85                  90                  95

AAG AAT CTT AAG ATA GAA GAC TCA GAT ACT TAC ATC TGT GAA GTG GGG     336
Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Gly
            100                 105                 110

GAC CAG AAG GAG GAG GTG CAA TTG CTA GTG TTC GGA TTG ACT GCC AAC     384
Asp Gln Lys Glu Glu Val Gln Leu Leu Val Phe Gly Leu Thr Ala Asn
        115                 120                 125

TCT GAC ACC CAC CTG CTT                                             402
Ser Asp Thr His Leu Leu
    130
```

SEQ ID NO.: 5
SEQUENCE TYPE: Nucleic acid
SEQUENCE LENGTH: 1374 bases

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: Y is C or T
M is A or C
S is G or C

ATGAACCGGG GAGTCCCTTT TAGGCACTTG CTTCTGGTGC TGCAACTGGC ACTCCTCCCA 60

GCAGCCACTC AGGGAAAGAA AGTGGTGCTG GGCAAGAAAG GGGACACAGT GGAACTGACC 120

TGTACAGCTT CCCAGAAGAA GAGCATACAA TTCCACTGGA AAAACTCCAA CCAGAYAAAG 180

ATTCTGGGAA ATCAGGGCTC CTTCTTAACT AAAGGTCCAT CCAAGCTGAA TGATCGCGYT 240

GACTCAAGAA GAAGCCTTTG GGACCAAGGA AACTTTMCCC TGATCATCAA GAATCTTAAG 300

ATAGAAGACT CAGATACTTA CATCTGTGAA GTGGGGGACC AGAAGGAGGA GGTGCAATTG 360

CTAGTGTTCG GATTGACTGC CAACTCTGAC ACCCACCTGC TTCAGGGGCA GAGCCTGACC 420

CTGACCTTGG AGAGCCCCCC TGGTAGTAGC CCCTCAGTGC AATGTAGGAG TCCAAGGGGT 480

AAAAACATAC AGGGGGGGAA GACCCTCTCC GTGTCTCAGC TGGAGCTCCA GGATAGTGGC 540

ACCTGGACAT GCACTGTCTT GCAGAACCAG AAGAAAGTGG AGTTCAAAAT AGACATCGTG 600

GTGCTAGCTT TCCAGAAGGC CTCCAGCATA GTCTATAAGA AAGAGGGGGA ACAGGTGGAG 660

TTCTCCTTCC CACTCGCCTT TACAGTTGAA AAGCTGACGG GCAGTGGCGA GCTGTGGTGG 720

CAGGCGGAGA GGGCTTCCTC CTCCAAGTCT TGGATCACCT TTGACCTGAA GAACAAGGAA 780

GTGTCTGTAA AACGGGTTAC CCAGGACCCT AAGCTCCAGA TGGGCAAGAA GCTCCCGCTC 840

CACCTCACCC TGCCCCAGGC CTTGCCTCAG TATGCTGGCT CTGGAAACCT CACCCTGGCC 900

CTTGAAGCGA AAACAGGAAA GTTGCATCAG GAAGTGAACC TCGTGGTGAT GAGAGCCACT 960

CAGCTCCAGA AAAATTTGAC CTGTGAGGTG TGGGGACCCA CCTCCCCTAA GCTGATGCTG 1020

AGCTTGAAAC TGGAGAACAA GGAGGCAAAG GTCTCGAAGC GGGAGAAGGC GGTGTGGGTG 1080

CTGAACCCTG AGGCGGGGAT GTGGCAGTGT CTGCTGAGTG ACTCGGGACA GGTCCTGCTG 1140

GAATCCAACA TCAAGGTTCT GCCCACATGG TCCACCCCGG TGCAGCCAAT GGCCCTGATT 1200

GTGCTGGGGG GCGTCGCCGG CCTCCTGCTT TTCATTGGGC TAGGCATCTT CTTCTGTGTC 1260

AGGTGCCGGC ACCGAAGGCG CCAAGCASAG CGGATGTCTC AGATCAAGAG ACTCCTCAGT 1320

GAGAAGAAGA CCTGCCAGTG CCCTCACCGG TTTCAGAAGA CATGTAGCCC CATT 1376

SEQ ID NO.: 6
SEQUENCE TYPE: Nucleic acid
SEQUENCE LENGTH: 1377 bases

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: Y is C or T
M is A or C

ATGAACCGGG GAGTCCCTTT TAGGCACTTG CTTCTGGTGC TGCAACTGGC GCTCCTCCCA 60

GCAGCCACTC AGGGAAAGAA AGTGGTGCTG GGCAAAAAAG GGGATACAGT GGAACTGACC 120

TGTACAGCTT CCCAGAAGAA GAGCATACAA TTCCACTGGA AAAACTCCAA CCAGAYAAAG 180

ATTCTGGGAA ATCAGGGCTC CTTCTTAACT AAAGGTCCAT CCAAGCTGAA TGATCGCGCT 240

GACTCAAGAA GAAGCCTTTG GGACCAAGGA AACTTTMCCC TGATCATCAA GAATCTTAAG 300

ATAGAAGACT CAGATACTTA CATCTGTGAA GTGGGGGACC AGAAGGAGGA GGTGCAATTG 360

CTAGTGTTCG GATTGACTGC CAACTCTGAC ACCCACCTGC TTCAGGGGCA GAGCCTGACC 420

CTGACCTTGG AGAGCCCCCC TGGTAGTAGC CCCTCAGTGC AATGTAGGAG TCCAAGGGGT 480

AAAAACATAC AGGGGGGGAA GACCCTCTCC GTGTCTCAGC TGGAGCTCCA GGATAGTGGC 540

ACCTGGACAT GCACTGTCTT GCAGAACCAG AAGAAGGTGG AGTTCAAAAT AGACATCGTG 600

GTGCTAGCTT TCCAGAAGGC CTCCAGCATA GTCTATAAGA AAGAGGGGGA ACAGGTGGAG 660

TTCTCCTTCC CACTCGCCTT TACAGTTGAA AAGCTGACGG GCAGTGGCGA GCTGTGGTGG 720

CAGGCGGAGA GGGCTTCCTC CTCCAAGTCT TGGATCACCT TTGACCTGAA GAACAAGGAA 780

GTGTCTGTAA AACGGGTTAC CCAGGACCCT AAGCTCCAGA TGGGCAAGAA GCTCCCGCTC 840

CACCTCACCC TGCCCCAGGC CTTGCCTCAG TATGCTGGCT CTGGAAACCT CACCCTGGCC 900

CTTGAAGCGA AAACAGGAAA GTTGCATCAG GAAGTGAACC TGGTGGTGAT GAGAGCCACT 960

CAGCTCCAGA AAAATTTGAC CTGTGAGGTG TGGGGACCCA CCTCCCCTAA GCTGATGCTG 1020

AGCTTGAAAC TGGAGAACAA GGAGGCAAAG GTCTCGAAGC GGGAGAAGGC GGTGTGGGTG 1080

CTGAACCCTG AGGCGGGGAT GTGGCAGTGT CTGCTGAGTG ACTCGGGACA GGTCCTGCTG 1140

GAATCCAACA TCAAGGTTCT GCCCACATGG TCCACCCCGG TGCAGCCAAT GGCCCTGATT 1200

GTGCTGGGGG GCGTCGCCGG CCTCCTGCTT TTCATTGGGC TAGGCATCTT CTTCTGTGTC 1260

AGGTGCCGGC ACCGAAGGCG CCAAGCAGAG CGGATGTCTC AGATCAAGAG ACTCCTCAGT 1320

GAGAAGAAGA CCTGCCAGTG CCCTCACCGG TTTCAGAAGA CATGTAGCCC CATTTGA 1377

SEQ ID NO.: 7
SEQUENCE TYPE: Nucleic acid
SEQUENCE LENGTH: 402 bases

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: Y is C or T
M is A or C

ATGAACCGGG GAGTCCCTTT TAGGCACTTG CTTCTGGTGC TGCAACTGGC GCTCCTCCCA 60

GCAGCCACTC AGGGAAAGAA AGTGGTGCTG GGCAAAAAAG GGGATACAGT GGAACTGACC 120

TGTACAGCTT CCCAGAAGAA GAGCATACAA TTCCACTGGA AAAACTCCAA CCAGAYAAAG 180

ATTCTGGGAA ATCAGGGCTC CTTCTTAACT AAAGGTCCAT CCAAGCTGAA TGATCGCGCT 240

GACTCAAGAA GAAGCCTTTG GGACCAAGGA AACTTTMCCC TGATCATCAA GAATCTTAAG 300

ATAGAAGACT CAGATACTTA CATCTGTGAA GTGGAGGACC AGAAGGAGGA GGTGCAATTG 360

CTAGTGTTCG GATTGACTGC CAACTCTGAC ACCCACCTGC TT 402

SEQ ID NO.: 8
SEQUENCE TYPE: Protein
SEQUENCE LENGTH: 458 amino acids

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: None

```
Met Asn Arg Gly Ile Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
                5                  10                  15

Ala Leu Leu Pro Ala Val Thr Gln Gly Lys Lys Val Val Leu Gly Lys
            20                  25                  30

Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Asn
        35                  40                  45

Thr Gln Phe His Trp Lys Asn Ser Asn Gln Ile Lys Ile Leu Gly Ile
    50                  55                  60

Gln Gly Leu Phe Leu Thr Lys Gly Pro Ser Lys Leu Ser Asp Arg Ala
65                  70                  75                  80

Asp Ser Arg Lys Ser Leu Trp Asp Gln Gly Cys Phe Ser Met Ile Ile
                85                  90                  95

Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Glu
            100                 105                 110

Asn Lys Lys Glu Glu Val Glu Leu Leu Val Phe Gly Leu Thr Ala Asn
        115                 120                 125

Ser Asp Thr His Leu Leu Glu Gly Gln Ser Leu Thr Leu Thr Leu Glu
    130                 135                 140

Ser Pro Pro Gly Ser Ser Pro Ser Val Lys Cys Arg Ser Pro Gly Gly
145                 150                 155                 160

Lys Asn Ile Gln Gly Gly Arg Thr Ile Ser Val Pro Gln Leu Glu Arg
                165                 170                 175

Gln Asp Ser Gly Thr Trp Thr Cys Thr Val Ser Gln Asp Gln Lys Thr
            180                 185                 190

Val Glu Phe Lys Ile Asp Ile Val Val Leu Ala Phe Gln Lys Ala Ser
        195                 200                 205

Ser Thr Val Tyr Lys Lys Glu Gly Glu Gln Val Glu Phe Ser Phe Pro
    210                 215                 220

Leu Ala Phe Thr Leu Glu Lys Leu Thr Gly Ser Gly Glu Leu Trp Trp
225                 230                 235                 240
```

Gln Ala Glu Arg Ala Ser Ser Ser Lys Ser Trp Ile Thr Phe Asp Leu
245 250 255

Lys Asn Lys Glu Val Ser Val Lys Arg Val Thr Gln Asp Pro Lys Leu
260 265 270

Gln Met Gly Lys Lys Leu Pro Leu His Leu Thr Leu Pro Gln Ala Leu
275 280 285

Pro Gln Tyr Ala Gly Ser Gly Asn Leu Thr Leu Ala Leu Glu Ala Lys
290 295 300

Thr Gly Lys Leu His Gln Glu Val Asn Leu Val Val Met Arg Ala Thr
305 310 315 320

Gln Leu Gln Lys Asn Leu Thr Cys Glu Val Trp Gly Pro Thr Ser Pro
325 330 335

Lys Leu Met Leu Ser Leu Lys Leu Glu Asn Lys Glu Ala Lys Val Ser
340 345 350

Lys Arg Glu Lys Ala Val Trp Val Leu Asn Pro Glu Ala Gly Met Trp
355 360 365

Gln Cys Leu Leu Ser Asp Ser Gly Gln Val Leu Leu Glu Ser Asn Ile
370 375 380

Lys Val Leu Pro Thr Trp Ser Thr Pro Val Gln Pro Met Ala Leu Ile
385 390 395 400

Val Leu Gly Gly Val Ala Gly Leu Leu Leu Phe Ile Gly Leu Gly Ile
405 410 415

Phe Phe Cys Val Arg Cys Arg His Arg Arg Arg Gln Ala Gln Arg Met
420 425 430

Ser Gln Ile Lys Arg Leu Leu Ser Glu Lys Lys Thr Cys Gln Cys Pro
435 440 445

His Arg Phe Gln Lys Thr Cys Ser Pro Ile
450 455

SEQ ID NO.: 9
SEQUENCE TYPE: Protein
SEQUENCE LENGTH: 458 amino acids

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: None

```
Met Asn Arg Gly Val Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
                  5                  10                  15

Ala Leu Leu Pro Ala Ala Thr Gln Gly Lys Lys Val Val Leu Gly Lys
             20                  25                  30

Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Ser
         35                  40                  45

Ile Gln Phe His Trp Lys Asn Ser Asn Gln Thr Lys Ile Leu Gly Asn
     50                  55                  60

Gln Gly Ser Phe Leu Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Val
 65                  70                  75                  80

Asp Ser Arg Arg Ser Leu Trp Asp Gln Gly Asn Phe Thr Leu Ile Ile
                 85                  90                  95

Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Gly
            100                 105                 110

Asp Gln Lys Glu Glu Val Gln Leu Leu Val Phe Gly Leu Thr Ala Asn
        115                 120                 125

Ser Asp Thr His Leu Leu Gln Gly Gln Ser Leu Thr Leu Thr Leu Glu
    130                 135                 140

Ser Pro Pro Gly Ser Ser Pro Ser Val Gln Cys Arg Ser Pro Arg Gly
145                 150                 155                 160

Lys Asn Ile Gln Gly Gly Lys Thr Leu Ser Val Ser Gln Leu Glu Leu
                165                 170                 175

Gln Asp Ser Gly Thr Trp Thr Cys Thr Val Leu Gln Asn Gln Lys Lys
            180                 185                 190

Val Glu Phe Lys Ile Asp Ile Val Val Leu Ala Phe Gln Lys Ala Ser
        195                 200                 205

Ser Ile Val Tyr Lys Lys Glu Gly Glu Gln Val Glu Phe Ser Phe Pro
    210                 215                 220

Leu Ala Phe Thr Val Glu Lys Leu Thr Gly Ser Gly Glu Leu Trp Trp
225                 230                 235                 240
```

Gln Ala Glu Arg Ala Ser Ser Ser Lys Ser Trp Ile Thr Phe Asp Leu
245 250 255

Lys Asn Lys Glu Val Ser Val Lys Arg Val Thr Gln Asp Pro Lys Leu
260 265 270

Gln Met Gly Lys Lys Leu Pro Leu His Leu Thr Leu Pro Gln Ala Leu
275 280 285

Pro Gln Tyr Ala Gly Ser Gly Asn Leu Thr Leu Ala Leu Glu Ala Lys
290 295 300

Thr Gly Lys Leu His Gln Glu Val Asn Leu Val Val Met Arg Ala Thr
305 310 315 320

Gln Leu Gln Lys Asn Leu Thr Cys Glu Val Trp Gly Pro Thr Ser Pro
325 330 335

Lys Leu Met Leu Ser Leu Lys Leu Glu Asn Lys Glu Ala Lys Val Ser
340 345 350

Lys Arg Glu Lys Ala Val Trp Val Leu Asn Pro Glu Ala Gly Met Trp
355 360 365

Gln Cys Leu Leu Ser Asp Ser Gly Gln Val Leu Leu Glu Ser Asn Ile
370 375 380

Lys Val Leu Pro Thr Trp Ser Thr Pro Val Gln Pro Met Ala Leu Ile
385 390 395 400

Val Leu Gly Gly Val Ala Gly Leu Leu Leu Phe Ile Gly Leu Gly Ile
405 410 415

Phe Phe Cys Val Arg Cys Arg His Arg Arg Arg Gln Ala Gln Arg Met
420 425 430

Ser Gln Ile Lys Arg Leu Leu Ser Glu Lys Lys Thr Cys Gln Cys Pro
435 440 445

His Arg Phe Gln Lys Thr Cys Ser Pro Ile
450 455

SEQ ID NO.: 10
SEQUENCE TYPE: Protein
SEQUENCE LENGTH: 458 amino acids

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: Glx is Glu or Gln
Xaa at position 59 is Thr or Ile
Xaa at position 80 is Val or Ala
Xaa at position 93 is Thr or Pro

```
Met Asn Arg Gly Val Pro Phe Arg His Leu Leu Val Leu Gln Leu
                5                  10                  15

Ala Leu Leu Pro Ala Ala Thr Gln Gly Lys Lys Val Val Leu Gly Lys
            20                  25                  30

Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Ser
        35                  40                  45

Ile Gln Phe His Trp Lys Asn Ser Asn Gln Xaa Lys Ile Leu Gly Asn
    50                  55                  60

Gln Gly Ser Phe Leu Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Xaa
65                  70                  75                  80

Asp Ser Arg Arg Ser Leu Trp Asp Gln Gly Asn Phe Xaa Leu Ile Ile
                85                  90                  95

Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Gly
            100                 105                 110

Asp Gln Lys Glu Glu Val Gln Leu Leu Val Phe Gly Leu Thr Ala Asn
        115                 120                 125

Ser Asp Thr His Leu Leu Gln Gly Gln Ser Leu Thr Leu Thr Leu Glu
    130                 135                 140

Ser Pro Pro Gly Ser Ser Pro Ser Val Gln Cys Arg Ser Pro Arg Gly
145                 150                 155                 160

Lys Asn Ile Gln Gly Gly Lys Thr Leu Ser Val Ser Gln Leu Glu Leu
                165                 170                 175

Gln Asp Ser Gly Thr Trp Thr Cys Thr Val Leu Gln Asn Gln Lys Lys
            180                 185                 190

Val Glu Phe Lys Ile Asp Ile Val Val Leu Ala Phe Gln Lys Ala Ser
        195                 200                 205

Ser Ile Val Tyr Lys Lys Glu Gly Glu Gln Val Glu Phe Ser Phe Pro
    210                 215                 220
```

Leu Ala Phe Thr Val Glu Lys Leu Thr Gly Ser Gly Glu Leu Trp Trp
225 230 235 240

Gln Ala Glu Arg Ala Ser Ser Ser Lys Ser Trp Ile Thr Phe Asp Leu
245 250 255

Lys Asn Lys Glu Val Ser Val Lys Arg Val Thr Gln Asp Pro Lys Leu
260 265 270

Gln Met Gly Lys Lys Leu Pro Leu His Leu Thr Leu Pro Gln Ala Leu
275 280 285

Pro Gln Tyr Ala Gly Ser Gly Asn Leu Thr Leu Ala Leu Glu Ala Lys
290 295 300

Thr Gly Lys Leu His Gln Glu Val Asn Leu Val Val Met Arg Ala Thr
305 310 315 320

Gln Leu Gln Lys Asn Leu Thr Cys Glu Val Trp Gly Pro Thr Ser Pro
325 330 335

Lys Leu Met Leu Ser Leu Lys Leu Glu Asn Lys Glu Ala Lys Val Ser
340 345 350

Lys Arg Glu Lys Ala Val Trp Val Leu Asn Pro Glu Ala Gly Met Trp
355 360 365

Gln Cys Leu Leu Ser Asp Ser Gly Gln Val Leu Leu Glu Ser Asn Ile
370 375 380

Lys Val Leu Pro Thr Trp Ser Thr Pro Val Gln Pro Met Ala Leu Ile
385 390 395 400

Val Leu Gly Gly Val Ala Gly Leu Leu Leu Phe Ile Gly Leu Gly Ile
405 410 415

Phe Phe Cys Val Arg Cys Arg His Arg Arg Arg Gln Ala Glx Arg Met
420 425 430

Ser Gln Ile Lys Arg Leu Leu Ser Glu Lys Lys Thr Cys Gln Cys Pro
435 440 445

His Arg Phe Gln Lys Thr Cys Ser Pro Ile
450 455

SEQ ID NO.: 11
SEQUENCE TYPE: Protein
SEQUENCE LENGTH: 134 amino acids

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: Xaa at position 59 is Thr or Ile
Xaa at position 80 is Val or Ala
Xaa at position 93 is Thr or Pro

```
Met Asn Arg Gly Val Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
                  5                  10                  15

Ala Leu Leu Pro Ala Ala Thr Gln Gly Lys Lys Val Val Leu Gly Lys
             20                  25                  30

Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Ser
         35                  40                  45

Ile Gln Phe His Trp Lys Asn Ser Asn Gln Xaa Lys Ile Leu Gly Asn
     50                  55                  60

Gln Gly Ser Phe Leu Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Xaa
 65                  70                  75                  80

Asp Ser Arg Arg Ser Leu Trp Asp Gln Gly Asn Phe Xaa Leu Ile Ile
                 85                  90                  95

Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Gly
            100                 105                 110

Asp Gln Lys Glu Glu Val Gln Leu Leu Val Phe Gly Leu Thr Ala Asn
        115                 120                 125

Ser Asp Thr His Leu Leu
    130
```

SEQ ID NO.: 12
SEQUENCE TYPE: Protein
SEQUENCE LENGTH: 458 amino acids

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: Xaa at position 59 is Thr or Ile
Xaa at position 93 is Thr or Pro

Met Asn Arg Gly Val Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
5 10 15

Ala Leu Leu Pro Ala Ala Thr Gln Gly Lys Lys Val Val Leu Gly Lys
20 25 30

Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Ser
35 40 45

Ile Gln Phe His Trp Lys Asn Ser Asn Gln Xaa Lys Ile Leu Gly Asn
50 55 60

Gln Gly Ser Phe Leu Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Ala
65 70 75 80

Asp Ser Arg Arg Ser Leu Trp Asp Gln Gly Asn Phe Xaa Leu Ile Ile
85 90 95

Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Glu
100 105 110

Asp Gln Lys Glu Glu Val Gln Leu Leu Val Phe Gly Leu Thr Ala Asn
115 120 125

Ser Asp Thr His Leu Leu Gln Gly Gln Ser Leu Thr Leu Thr Leu Glu
130 135 140

Ser Pro Pro Gly Ser Ser Pro Ser Val Gln Cys Arg Ser Pro Arg Gly
145 150 155 160

Lys Asn Ile Gln Gly Gly Lys Thr Leu Ser Val Ser Gln Leu Glu Leu
165 170 175

Gln Asp Ser Gly Thr Trp Thr Cys Thr Val Leu Gln Asn Gln Lys Lys
180 185 190

Val Glu Phe Lys Ile Asp Ile Val Val Leu Ala Phe Gln Lys Ala Ser
195 200 205

Ser Ile Val Tyr Lys Lys Glu Gly Glu Gln Val Glu Phe Ser Phe Pro
210 215 220

Leu Ala Phe Thr Val Glu Lys Leu Thr Gly Ser Gly Glu Leu Trp Trp
225 230 235 240

Gln Ala Glu Arg Ala Ser Ser Ser Lys Ser Trp Ile Thr Phe Asp Leu
245 250 255

Lys Asn Lys Glu Val Ser Val Lys Arg Val Thr Gln Asp Pro Lys Leu
260 265 270

Gln Met Gly Lys Lys Leu Pro Leu His Leu Thr Leu Pro Gln Ala Leu
275 280 285

Pro Gln Tyr Ala Gly Ser Gly Asn Leu Thr Leu Ala Leu Glu Ala Lys
290 295 300

Thr Gly Lys Leu His Gln Glu Val Asn Leu Val Val Met Arg Ala Thr
305 310 315 320

Gln Leu Gln Lys Asn Leu Thr Cys Glu Val Trp Gly Pro Thr Ser Pro
325 330 335

Lys Leu Met Leu Ser Leu Lys Leu Glu Asn Lys Glu Ala Lys Val Ser
340 345 350

Lys Arg Glu Lys Ala Val Trp Val Leu Asn Pro Glu Ala Gly Met Trp
355 360 365

Gln Cys Leu Leu Ser Asp Ser Gly Gln Val Leu Leu Glu Ser Asn Ile
370 375 380

Lys Val Leu Pro Thr Trp Ser Thr Pro Val Gln Pro Met Ala Leu Ile
385 390 395 400

Val Leu Gly Gly Val Ala Gly Leu Leu Leu Phe Ile Gly Leu Gly Ile
405 410 415

Phe Phe Cys Val Arg Cys Arg His Arg Arg Arg Gln Ala Glu Arg Met
420 425 430

Ser Gln Ile Lys Arg Leu Leu Ser Glu Lys Lys Thr Cys Gln Cys Pro
435 440 445

His Arg Phe Gln Lys Thr Cys Ser Pro Ile
450 455

SEQ ID NO.: 13
SEQUENCE TYPE: Protein
SEQUENCE LENGTH: 134 amino acids

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: None

```
Met Asn Arg Gly Ile Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
                  5                  10                  15

Ala Leu Leu Pro Ala Val Thr Gln Gly Lys Lys Val Val Leu Gly Lys
             20                  25                  30

Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Asn
         35                  40                  45

Thr Gln Phe His Trp Lys Asn Ser Asn Gln Ile Lys Ile Leu Gly Ile
     50                  55                  60

Gln Gly Leu Phe Leu Thr Lys Gly Pro Ser Lys Leu Ser Asp Arg Ala
 65                  70                  75                  80

Asp Ser Arg Lys Ser Leu Trp Asp Gln Gly Cys Phe Ser Met Ile Ile
                 85                  90                  95

Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Glu
            100                 105                 110

Asn Lys Lys Glu Glu Val Glu Leu Leu Val Phe Gly Leu Thr Ala Asn
        115                 120                 125

Ser Asp Thr His Leu Leu
    130
```

SEQ ID NO.: 14
SEQUENCE TYPE: Protein
SEQUENCE LENGTH: 134 amino acids

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: None

```
Met Asn Arg Gly Val Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
                  5                  10                  15

Ala Leu Leu Pro Ala Ala Thr Gln Gly Lys Lys Val Val Leu Gly Lys
             20                  25                  30

Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Ser
         35                  40                  45

Ile Gln Phe His Trp Lys Asn Ser Asn Gln Thr Lys Ile Leu Gly Asn
     50                  55                  60

Gln Gly Ser Phe Leu Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Val
 65                  70                  75                  80

Asp Ser Arg Arg Ser Leu Trp Asp Gln Gly Asn Phe Thr Leu Ile Ile
                 85                  90                  95

Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Gly
            100                 105                 110

Asp Gln Lys Glu Glu Val Gln Leu Leu Val Phe Gly Leu Thr Ala Asn
        115                 120                 125

Ser Asp Thr His Leu Leu
    130
```

SEQ ID NO.: 15
SEQUENCE TYPE: Protein
SEQUENCE LENGTH: 134 amino acids

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: Xaa at position 59 is Thr or Ile
Xaa at position 93 is Thr or Pro

Met Asn Arg Gly Val Pro Phe Arg His Leu Leu Val Leu Gln Leu
5 10 15

Ala Leu Leu Pro Ala Ala Thr Gln Gly Lys Lys Val Val Leu Gly Lys
20 25 30

Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Ser
35 40 45

Ile Gln Phe His Trp Lys Asn Ser Asn Gln Xaa Lys Ile Leu Gly Asn
50 55 60

Gln Gly Ser Phe Leu Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Ala
65 70 75 80

Asp Ser Arg Arg Ser Leu Trp Asp Gln Gly Asn Phe Xaa Leu Ile Ile
85 90 95

Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Glu
100 105 110

Asp Gln Lys Glu Glu Val Gln Leu Leu Val Phe Gly Leu Thr Ala Asn
115 120 125

Ser Asp Thr His Leu Leu
130

SEQ ID NO.: 16
SEQUENCE TYPE: Protein
SEQUENCE LENGTH: 134 amino acids

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: None

Met Asn Arg Gly Ile Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
5 10 15

Ala Leu Leu Pro Ala Val Thr Gln Gly Lys Lys Val Val Leu Gly Lys
20 25 30

Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Asn
35 40 45

Thr Gln Phe His Trp Lys Asn Ser Asn Gln Ile Lys Ile Leu Gly Ile
50 55 60

Gln Gly Leu Phe Leu Thr Lys Gly Pro Ser Lys Leu Ser Asp Arg Ala
65 70 75 80

Asp Ser Arg Lys Ser Leu Trp Asp Gln Gly Cys Phe Ser Met Ile Ile
85 90 95

Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Glu
100 105 110

Asn Lys Lys Glu Glu Val Glu Leu Leu Val Phe Gly Leu Thr Ala Asn
115 120 125

Ser Asp Thr His Leu Leu
130

SEQ ID NO.: 17
SEQUENCE TYPE: Protein
SEQUENCE LENGTH: 134 amino acids

STRANDEDNESS: Single
TOPOLOGY: Linear

FEATURES: None

```
Met Asn Arg Gly Val Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
                5                  10                  15

Ala Leu Leu Pro Ala Ala Thr Gln Gly Lys Lys Val Val Leu Gly Lys
            20                  25                  30

Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Ser
        35                  40                  45

Ile Gln Phe His Trp Lys Asn Ser Asn Gln Thr Lys Ile Leu Gly Asn
    50                  55                  60

Gln Gly Ser Phe Leu Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Val
65                  70                  75                  80

Asp Ser Arg Arg Ser Leu Trp Asp Gln Gly Asn Phe Thr Leu Ile Ile
                85                  90                  95

Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Gly
            100                 105                 110

Asp Gln Lys Glu Glu Val Gln Leu Leu Val Phe Gly Leu Thr Ala Asn
        115                 120                 125

Ser Asp Thr His Leu Leu
    130
```

C195-09.WP5

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 414 178 A3**

(12) **EUROPEAN PATENT APPLICATION**

(21) Application number: **90115877.4**

(22) Date of filing: **18.08.90**

(51) Int. Cl.5: **C12N 15/12**, C12N 15/62, C12N 15/13, C12P 21/02, C12Q 1/70, A61K 37/02, G01N 33/569

(30) Priority: **23.08.89 US 397782**

(43) Date of publication of application: **27.02.91 Bulletin 91/09**

(84) Designated Contracting States: **AT BE CH DE DK ES FR GB GR IT LI LU NL SE**

(88) Date of deferred publication of the search report: **13.11.91 Bulletin 91/46**

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(54) Non-human primate CD4 polypeptides and human CD4 molecules capable of being glycosylated.

(57) The invention relates to substantially pure non-human primate CD4, and fragments thereof which bind to HIV or SIV gp120. The invention also relates to gp120 binding molecules related to human CD4 but which may exist in glycosylated form.

The invention also relates to fusion proteins which comprise the CD4 molecules of the invention, or fragments thereof, and an immunoglobulin light or heavy chain, wherein the variable region of the light or heavy chain has been replaced with CD4 or fragment thereof which is capable of binding to gp120. The invention also relates to fusion proteins comprising the CD4 molecules of the invention and a cytotoxic polypeptide.

The invention also relates to an immunoglobulin-like molecules comprising the fusion proteins of the invention together with an immunoglobulin light or heavy chain.

The invention also relates to methods of treating HIV or SIV infection comprising administering the CD4 molecules of the invention, glycoproteins, fragments thereof, fusion proteins or immunoglobulin-like molecules of the invention to an animal.

The invention also relates to assays for HIV or SIV comprising contacting a sample suspected of containing HIV or SIV gp120 with the CD4 molecules of the invention, fragments thereof, glycoproteins, immunoglobulin-like molecules, or fusion proteins of the invention, and detecting whether a complex is formed.

The invention also relates to nucleic acid molecules which specify the proteins, glycoproteins and fusion proteins of the invention as well as vectors and transformed hosts.

EP 0 414 178 A3

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 11 5877

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Y	CELL, vol. 42, August 1985, pages 93-104; P.J. MADDON et al.: "The isolation and nucleotide sequence of a cDNA encoding the T cell surface protein T4: A new member of the immunoglobulin gene family" * Summary; Introduction; Results; especially figure 6 *	1-30	C 12 N 15/12 C 12 N 15/62 C 12 N 15/13 C 12 P 21/02 C 12 Q 1/70 A 61 K 37/02 G 01 N 33/569
Y	EP-A-0 314 317 (GENENTECH, USA)(03-05-1989) * Page 3, lines 11-38; pages 4,5; claims *	1-30	
Y	WO-A-8 801 304 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK, US)(25-02-1988) * Summary; pages 16-23; figure 6A; claims *	1-30	
A	NATURE, vol. 331, no. 6151, 7th January 1988, pages 78-81; R.E. HUSSEY et al.: "A soluble CD4 protein selectively inhibits HIV replication and syncytium formation" * The whole document *	1-30	
A	NATURE, vol. 331, no. 6151, 7th January 1988, pages 84-86; A. TRAUNECKER et al.: "Soluble CD4 molecules neutralize human immunodeficiency virus type 1" * The whole document *	1-30	TECHNICAL FIELDS SEARCHED (Int. Cl.5) C 07 K C 12 N
A	NATURE, vol. 331, no. 6151, 7th January 1988, pages 82-84; K.C. DEEN et al.: "A soluble form of CD4 (T4) protein inhibits AIDS virus infection" * The whole document *	1-30	
A	NATURE, vol. 331, no. 6151, 7th January 1988, pages 76-78; R.A. FISHER et al.: "HIV infection is blocked in vitro by recombinant soluble CD4" * The whole document *	1-30	

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	02 September 91	NAUCHE S.A.

CATEGORY OF CITED DOCUMENTS

X: particularly relevant if taken alone
Y: particularly relevant if combined with another document of the same catagory
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention

E: earlier patent document, but published on, or after the filing date
D: document cited in the application
L: document cited for other reasons

&: member of the same patent family, corresponding document